Fungal exopolysaccharides: properties, sources, modifications, and biomedical applications

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Abstract

Fungal exopolysaccharides (EPSs) are natural biopolymers with diverse potential applications in the biomedical, packaging, cosmetic, and food industries. Fungal EPSs are easy to extract and purify polysaccharides that are biodegradable, biocompatible, with low immunogenicity, bioadhesion ability, antibacterial activity, and contain different reactive groups such as hydroxyl, carboxyl, and amine for chemical modifications. Despite fast progress in identifying and characterization fungal EPSs for biomedical applications, i.e., wound healing, drug, and gene delivery, only a few products have been commercialized based on fungal EPSs. This review critically discusses potential biomedical applications of fungi sourced EPSs in tissue engineering (TE), drug and gene delivery.

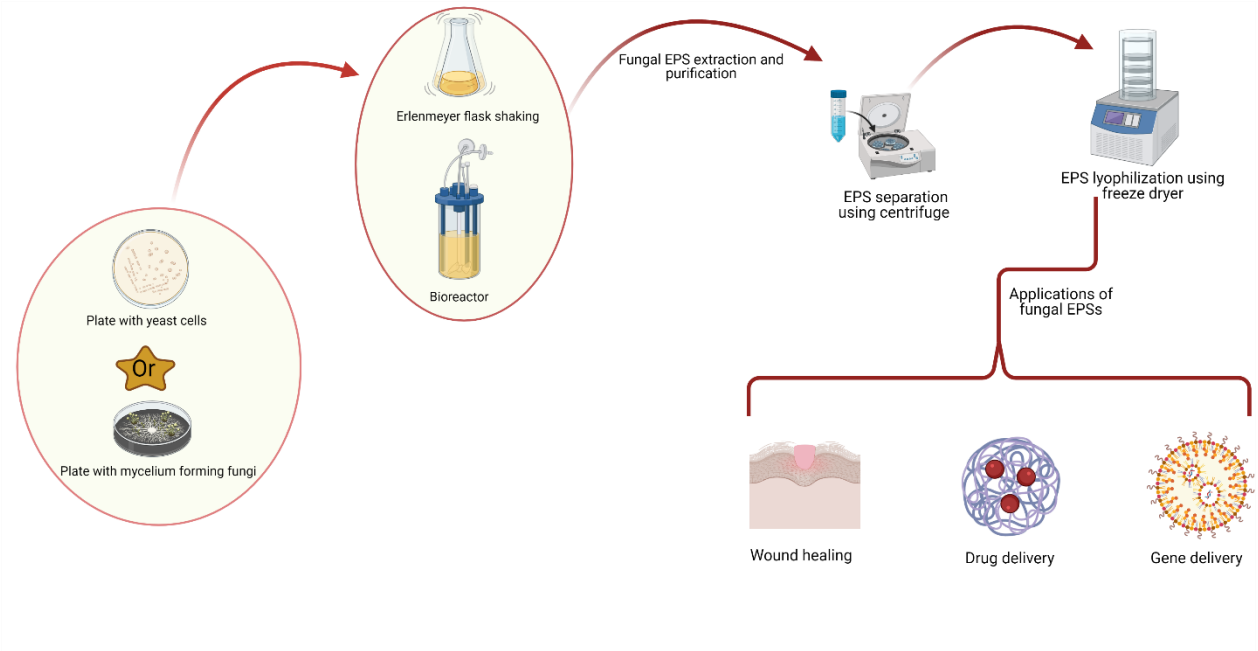
Keywords: Exopolysaccharide; Tissue engineering; Drug delivery, Gene delivery

Chemical compounds studied in this article: Chitosan (PubChem CID: 71853); Lentinan (PubChem CID: 37723); Pullulan (PubChem CID: 439586); Schizophyllan (PubChem CID: 24777); Scleroglucan (PubChem CID: 131750928).

Abbreviation list:

aECM, acellular extracellular matrix; anti-TB, anti-tuberculosis, AuNPs, gold nanoparticles; BC, bacterial cellulose; βG, β-Glucan; BRMs, biological response modifiers; CM-Scl, carboxymethyl Scl; CHP, cholesterol-modified PUL; CGC, chitin–glucan complex; ChGC, chitosan–glucan complex; CSPN, chitosan-PUL-silver-nanocomposite; CR3, complement receptor 3; DB, degree of branching; DCs, dendritic cells; DEX-MA, dextran methacrylate; D-Glcp, D-glucopyranose; DFUs, diabetic foot ulcers; DBAP-PO, dibutylaminopropyl carbamate pullulan octanoate; DEAE, diethyl amino ethyl; DDSs, drug delivery systems; EPSs, exopolysaccharides; GPC, gel permeation chromatography; GPs, glucan particles; Human Hepatocellular Carcinoma; HPCys-Pul, Hydroxypropyl cyclosophoraose-PUL; IEC, ion exchange chromatography; IPN, interpenetrating polymeric network; LacCer, lactosylceramide; LAS, Lasiodiplodan; MWs, molecular weights; MGB, myoglobin; NPs, nanoparticles; OXPL, oxidized PUL; PABA-QP, para-aminobenzoic acid-quat188-PUL; PAMP, pathogen-associated molecular pattern; PRRs, pattern recognition receptors; PDGF, Platelet-derived growth factor; PDA, polydopamine; PEI, polyethyleneimine; PSs, polysaccharides; PVA, polyvinyl alcohol; PUL, pullulan; PHG, PUL hydrogel; PUL-MNs, PUL microneedle; SC, Sacchachitin; SCNF, sacchachitin nanofibers; SPG, schizophyllan; Scl, scleroglucan; Smf, submerged fermentation; STMP, sodium trimetaphosphate; SBG, soluble βG; TEMPO, 2,2,6,6-Tetramethylpiperidyl-1-Oxyl; TE, tissue engineering; TF, monocyte tissue factor; TGF-β, Transforming growth factor beta; VEGF, Vascular endothelial growth factor; VitB12, vitamin B12

Graphical abstract



1. Introduction

Polysaccharides (PSs) such as alginate, chitosan, hyaluronic acid, cellulose, etc. constitute an important example of biopolymers that are recovered from plants, seaweeds, bacteria, fungi, etc. (Castillo, Valdez, & Farina, 2015; Cosenza, Navarro, Ponce, & Stortz, 2017; Vanina A. Cosenza, Navarro, & Stortz, 2017; Hamidi et al., 2019; Luft, Confortin, Todero, Zabot, & Mazutti, 2020; Shanmugam & Abirami, 2019; Sugumaran & Ponnusami, 2017) and have been developed into hydrogels, aerogels, films, membranes, fibers, and sponges suitable for different tissue engineering (TE) and biomedical applications (Cohen & Merzendorfer, 2019; Safarzadeh Kozani et al., 2021). Among the natural sources of PSs, the microbial exopolysaccharides (EPSs) such as alginate, chitosan, pullulan (PUL), scleroglucan (Scl), xanthan gum, bacterial cellulose, etc., have favorable biological and mechanical properties for TE scaffold fabrication (Smelcerovic, Knezevic-Jugovic, & Petronijevic, 2008).

In comparison to marine or plant-based PSs, microbial EPSs production only require a few days, do not compete with production lands, agro-industrial waste can be used as their feed, and are frequently simple to extract and purify (Castillo et al., 2015; Elsehemy et al., 2020) with typical production yields ranging from 0.0022–100 g/L (Castillo et al., 2015; Freitas, Torres, & Reis, 2017). Some fungi such as *Aureobasidium pullulans* can produce more than 40 g/L EPS (pullulan) under elementary production states (Luft et al., 2020). Microbial EPSs yield varies based on the type of the strain and factors such as the conditions of the fermentation parameters such as pH, temperature, etc. (Elsehemy et al., 2020; Okoro, Gholipour, Sedighi, Shavandi, & Hamidi, 2021; Saadat, Khosroushahi, & Gargari, 2021; Shanmugam & Abirami, 2019).

While bacteria and fungi are the most common sources of microbial EPSs (M. C. S. Barcelos, K. A. C. Vespermann, F. M. Pelissari, & G. Molina, 2020; Hamidi et al., 2020; S. Mahapatra & D. Banerjee, 2013; Saadat et al., 2021), fungal EPSs have received less attention (T Coviello, Grassi, Rambone, & Alhaique, 2001; S. Mahapatra & D. Banerjee, 2013). Recognizing the importance and benefits of fungal EPSs (Fig. 1A), the present work will present a comprehensive literature review, with an emphasis on studies published in the last decade and explore fungal EPSs production and their potential for biomedical applications such as anti-inflammatory effects (Barbieri et al., 2017; Rajasekar, Selvakumar, Periasamy, & Raaman, 2008), improvement of the immune system (Rajasekar et al., 2008; Zhao, Chen, & He, 2018), anticancer actions (Barbieri et al., 2017; S. Mahapatra & D. Banerjee, 2013; Rajasekar et al., 2008; Zhao et al., 2018) influence on the cardiovascular system (S. Mahapatra & D. Banerjee, 2013; Rajasekar et al., 2008), and treatment of hypercholesterolemia and diabetes (Asadi, Barshan-Tashnizi, Hatamian-

Zarmi, Davoodi-Dehaghani, & Ebrahimi-Hosseinzadeh, 2021; S. Mahapatra & D. Banerjee, 2013; Rajasekar et al., 2008). The present review will also highlight perspectives for novel EPSs applications.

2. General properties of fungal EPSs

Fungal cells produce EPS during the whole growth phase (Osińska-Jaroszuk et al., 2015; Sardari et al., 2017). Fungal EPSs comprise monomeric units such as D-xylose, fucose, etc. The fungal EPSs are diverse in the monosaccharide linkage patterns, monosaccharide units, molecular mass, branching degree, glycosidic bond, and conformation (Asadi et al., 2021; Gong et al., 2020). In addition, EPSs comprised of similar monosaccharide entities produced by various fungi had distinct molecular weights (MWs) (Elsehemy et al., 2020). Fungal EPSs may present several structures such as extracellular glucan characterized by β-(1,3)-D-pyran glycosidic bonding, α-monosaccharides configuration with predominantly 1, 6 linkages, or α-(1,6) maltotriose sub-units produced from *Lachnum* sp. YM2261, *Aspergillus parasiticus*, and *Cryphonectria parasitica*, respectively (Ye et al., 2012; Ruperez & Leal, 1981; Forabosco et al., 2006).

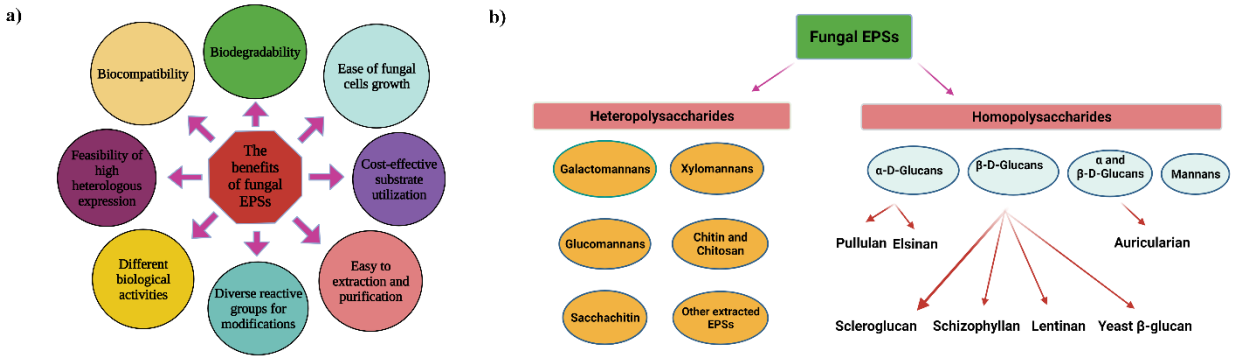


Fig. 1. a) Benefits of fungal EPSs for biomedical applications. **b)** The schematic diagram represents the classification of the fungal EPSs based on the type of sugar monomers, including the names of some essential fungal EPSs.

The EPS properties (i.e., chemical composition) depend largely on the agitation intensity of bioreactors, and depending on the bioreactor employed in fermentation; the EPSs may have different sugar units with varying MWs (C. P. Xu, Kim, Hwang, & Yun, 2006). For example, using a stirred-tank bioreactor leads to the EPS production by *Paecilomyces tenuipes* C240 composed of glucose and mannose units. In contrast, using airlift bioreactors resulted in EPSs with glucose and arabinose units with different MWs (C. P. Xu et al., 2006). There was no significant difference in the yield of EPS using the stirred-tank and airlift bioreactors (C. P. Xu et al., 2006). In the other study by Kim et al., by changing the agitation speed (50-300 rpm) and aeration rate (0.5-2 vvm), variations in the structural

compositions of the EPS with antioxidant activity from *Ganoderma resinaceum* were reported, with monosaccharides of fructose, mannose, xylose, glucose, and galactose, retained (Kim, et al, 2006). Indeed, it was observed that there was a clear variance in the composition (%) of each of monosaccharides resulting in different antioxidative activities. So careful control of the agitation and aeration is important to confirm the quality of the biological activity of the produced EPSs. The reason for the variation in EPSs characteristics and their monosaccharide compositions due to the differences in the environmental conditions is still unknown. However, it has been reported that bioreactor hydrodynamics such as the micromixing phenomenon and cell metabolic activity can improve oxygen availability and affect mass transfer characteristics (Lazaridou, Roukas, Biliaderis, & Vaikousi, 2002; Xu, Kim, Hwang, & Yun, 2006) which may impact on EPSs properties. Kim et al., concluded that a high oxygen transfer would be suitable for most mushrooms in submerged cultures for EPS synthesis (Kim, et al, 2006).

3. Classification of fungal EPSs

Fungal EPSs are secreted into the extracellular medium in the form of biofilm or capsules (M. C. Barcelos, K. A. Vespermann, F. M. Pelissari, & G. Molina, 2020; Kagimura, da Cunha, Barbosa, Dekker, & Malfatti, 2015). Fungal EPSs are characterized based on the presence or absence of uronic acid, as acidic (e.g., the EPS with wound healing activity from *Cryptococcus laurentii* 70766 (DSMZ collection) (Smirnou et al., 2014)) or neutral (e.g., PUL, β-glucans (βGs), etc.), respectively (Kalia, 2016). Most fungal EPSs are linear hetero PSs (Fig. 1B) consisting of repeating units of different monosaccharides such as pentoses, hexoses, amino sugars, and uronic acids (Shanmugam & Abirami, 2019).

Hyperbranched fungal EPSs, i.e., lentinan from *Lentinus edodes* and schizophyllan (SPG) from *Schizophyllum commune* have numerous functional groups with high density and low viscosity (L. Chen et al., 2019). The lentinan is a known bioactive hyperbranched fungal EPS, which is a β-(1→3,6)-D-glucan having two β-(1→6)-D-Glcp branches for every five β-(1→3)-D-Glcp linear linkages. Likewise, SPG was described to be also composed of β-(1→3,6)-D-glucan with a higher degree of branching (DB) (50%) that possess one β-(1→6)-D-Glcp branching for every three β-(1→3)-D-Glcp in the backbone. Although branches in EPSs from *L. edodes* and *S. commune* contain only the terminal β-D-Glcp, both have the triple-helix pattern and numerous bioactivities, e.g., anti-cancer and immunomodulatory effects (L. Chen et al., 2019).

4. Types and sources of fungal EPSs

4.1. Yeast-derived EPSs

EPSs are made by numerous yeast cells, for instance, strains of *Bullera* (Vlaev et al., 2013), *Candida* (Gonçalves, Del Bel Cury, de Vasconcellos, Cury, & da Silva, 2015), *Cryptococcus* (Rusinova-Videva, Pavlova, & Georgieva, 2011), *Debaryomyces* (Choudhury, Saluja, & Prasad, 2011), *Lipomyces* (Ragavan & Das, 2019), *Pichia* (Saadat, Khosroushahi, Movassaghpour, Talebi, & Gargari, 2020), *Pseudozyma* (K. N. Kim et al., 2020), *Rhodotorula* (Hamidi et al., 2020; Mirzaei Seveiri et al., 2019), *Rhodosporidium* (Mirzaei Seveiri et al., 2020) and *Sporobolomyces* (K. Pavlova, Zlatanov, Antova, Angelova-Romova, & Georgieva, 2012) genera that are generally regarded as safe and may seldomly cause opportunistic infections in humans (Gientka, Błażejak, Stasiak-Różańska, & Chlebowska-Śmigiel, 2015). EPS production is associated with yeast metabolism with factors such as the formulation of the culture medium and fermentation conditions such as pH, temperature, and oxygen level influencing and their physical and structural properties (Gientka et al., 2015).

Glucans, mannans, and chitin are major PSs in the yeasts' cell wall, complex in composition and structure (K. I. Pavlova, 2014; Saadat et al., 2021). Phosphate or other chemical groups, such as uronic acid, have also been determined in yeasts' EPSs. The chemical constituents are impacted by culture settings, principally carbon and nitrogen supplies, besides stress issues (Gientka et al., 2015). For example, the crude EPS preparation produced by *C. laurentii* under conditions of salt stress, i.e., in media containing 10% NaCl, facilitated the production of EPS containing 13.8% and 44% of protein and mannose, respectively relative to the EPS obtained at similar conditions in the absence of NaCl, with 33 % and 60 % of protein and mannose produced, respectively (Elinov, Gurina, & Ananeva, 1995).

4.2. Filamentous fungi-derived EPSs

Cell wall PSs from filamentous fungi such as *Sclerotium rolfsii*, *Botryosphaeria rhodian*, and *Elsinoe leucospila* were shown to possess biological activities such as antioxidant, anti-inflammatory, immunomodulatory, antinociceptive, anti-tumor, and hypoglycemic properties (Junior et al., 2020). In addition to PSs, the EPSs are important part of the extracellular biofilm matrix of filamentous fungi capable of diffusing to the liquid phase of the fermentation media (Junior et al., 2020). PSs in *Ascomycetes* are predominantly hetero-PSs, and glucose, mannose, galactose are often presented (Q. Wang, Wang, Xu, & Ding, 2017). However, PSs from *Basidiomycetes* are more

complex, mainly in respect to monosaccharide composition and molar ratios of hetero-PSs (Ruthes, Smiderle, & Iacomini, 2016; Q. Wang et al., 2017).

4.3. Major fungal EPSs

In this section, sources, properties, and potential applications of some important fungal EPSs are discussed.

4.3.1. β-glucans (βGs)

Non-cellulosic β-D-glucans are a well-studied class of EPSs (Cohen & Merzendorfer, 2019) that can be produced extracellularly when cultured under submerged fermentation (SmF) settings. Examples are lentinan, SPG, Scl, botryosphaeran, and lasiodiplodan from *L. edodes*, *S. commune*, *S. rolfsii*, *B. rhodina* and *L. theobromae*, respectively (Cohen & Merzendorfer, 2019; Goodridge, Wolf, & Underhill, 2009; Stalhberger et al., 2014; Synytsya & Novák, 2013; Wasser, 2014).

Yeast sourced βGs typically contain mixtures of linear β (1→3) backbones, β (1→6) branches, and straight residue chains (Manners, Masson, & Patterson, 1973). Some fungal glucans (C. Chen et al., 2014; He et al., 2020; Yifeng Wang, Zhang, Li, Hou, & Zeng, 2004; Zeković, Kwiatkowski, Vrvić, Jakovljević, & Moran, 2005; M. Zhang, Cheung, Zhang, Chiu, & Ooi, 2004) such as the βG from the sclerotia of *Pleurotus tuber-regium* were chemically modified using carboxymethylation (M. Zhang et al., 2004) to obtain water-soluble derivatives (Synytsya & Novák, 2013).

Branched and linear β-D-glucans are among the most studied fungal glucans, and they are recognized as physiologically active compounds referred to as biological response modifiers (BRMs). These glucans may be used as remedies in bacterial, viral, or protozoal infections, and have application in antitumor drugs (Geller & Yan, 2020; Steimbach et al., 2020; Synytsya & Novák, 2013). The cellular response by βGs is due to their interaction with Dectin-1, complement receptor 3 (CR3), scavenger receptors, and lactosylceramide (LacCer), which are pattern recognition receptors (PRRs), that can trigger signal transduction in polymorphonuclear phagocytes (e.g., macrophages, monocytes, dendritic cells, and natural killer cells) and neutrophils (Brown & Gordon, 2001; Chan, Chan, & Sze, 2009; Taylor et al., 2007). The performance of the PRRs depends on the cell characteristics; for example, βG induced neutrophil modulation is largely CR3 dependent, while Dectin-1 is the most crucial βG receptor on macrophages (Baert, Sonck, Goddeeris, Devriendt, & Cox, 2015; Han, Baruah, Cox, Vanrompay, & Bossier, 2020). βG binding to the lectin site of the CR3 on phagocytes and NK cells enables the activation of

receptors to enhance the cytotoxicity against iC3b-opsonized target cells (Ross, Větvička, Yan, Xia, & Větvičková, 1999; Vetvicka, Thornton, & Ross, 1996). Recognition of βG by Dectin-1 on macrophages activates the downstream signaling pathway and Dectin-1 triggers phagocytosis, ROS generation, microbial killing, and cytokine production (Han et al., 2020; Sato et al., 2006).

DB of about 0.2–0.3 has the highest antitumor property as presented by lentinan, SPG, or yeast β-D-glucan (Synytsya & Novák, 2013). Notably, βGs from diverse sources differ in their structure, physical properties, binding affinity to receptors, and consequently biological functions, which mechanisms are unclear (Han et al., 2020). As presented in Fig. 1B, several fungal βGs reported in the literature, which as examples, Scleroglucan (Scl) and Lasiodiplodan are explained in the following sections:

4.3.1.1. Scleroglucan (Scl)

The two leading species for Scl synthesis are *S. glucanicum* and *S. rolfsii* (Survase, Saudagar, Bajaj, & Singhal, 2007). Scl consists of β-(1→3)-linked glucose with a β-(1→6)-glycosyl branch on every third unit. Its highest yield (66.6 g/l) extracted from *S. rolfsii* WSH-G01 was gained by a two-dose fed-batch mode (Zeng, Wang, Shan, Yu, & Zhou, 2021). Commercialization of Scl was first done in the 70s, which is now existing under several trademarks (e.g., Clearogel, Polytetran, Scl, and Actigum) for different uses, e.g., in hair control compositions (J. Park & Khan, 2009; Schmid, Meyer, & Sieber, 2011), oil recovery, drug delivery, and as an emulsifier (M. C. S. Barcelos et al., 2020; Luft et al., 2020). Scl's potential industrial importance is due to its favorable water-solubility, viscosity, stability, biocompatibility properties, etc. (M. C. S. Barcelos et al., 2020). Relevant activities of Scl for health comprise hypocholesterolemic, hypoglycemic, health-promoting outcomes, antioxidant, antiviral, and anti-obesity effects (N. A. Castillo et al., 2015; Survase et al., 2007). Also resembling other βGs, Scl shows an antineoplastic effect, although it is more efficient than other PSs, e.g., curdlan and yeast β-glucan (Survase et al., 2007; Y Wang & McNeil, 1995).

4.3.1.2. Lasiodiplodan (LAS)

LAS from *L. theobromae* MMPI, as a linear (1,6) β-glucan, was first described in 2008. It displays biological functions such as antioxidant, and transaminase activities (Cohen & Merzendorfer, 2019; Nissola et al., 2021). Additionally, LAS presents protective activity against doxorubicin-induced DNA damage (Cohen & Merzendorfer, 2019; Nissola et al., 2021). The numerous biological possessions of LAS and the ease of production by SmF (like other fungal EPSs) and retrieval from the fermentation broth free of cells, present it a biomolecule

desirable for commercial use (Cohen & Merzendorfer, 2019; Nissola et al., 2021). LAS has remarkable rheological properties, e.g., high apparent viscosity at 25℃ (Cohen & Merzendorfer, 2019). Recently Nissola et al. evaluated the wound healing potential of a hydrogel including LAS on the Wistar rats. The hydrogel stimulated cell re-epithelialization and proliferation and stimulated collagen fiber generation (Nissola et al., 2021). Chemical changes in the LAS structure by O-acetylation, carboxymethylation, phosphorylation, or sulfonylation (Fig. 2), were revealed to be potential methods for altering the chemical and biological characteristics (Cohen & Merzendorfer, 2019).

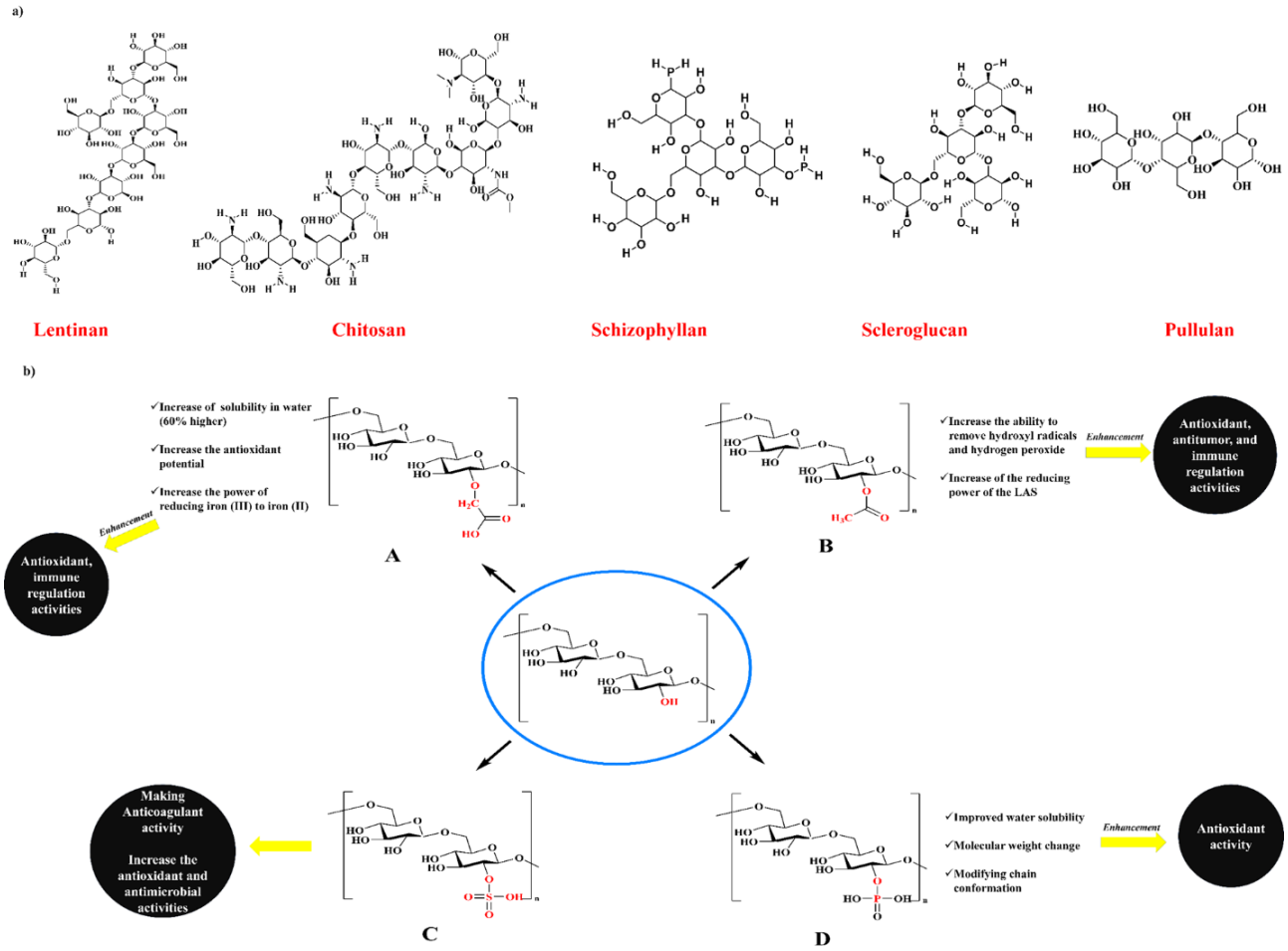


Fig. 2. a) Chemical structures of five major fungal EPSs (as mentioned in the list of the chemical compounds). **b**) Structural representation of Lasiodiplodan+ its derivatives by carboxymethylation **(A)**, acetylation **(B)**, sulfation **(C)**, and phosphorylation **(D)**.

For example, the O-Acetylation of LAS was described by Sánchez et al. (Luna et al., 2018). In the study, O-acetylated derivatives of LAS were produced by using acetic anhydride as the derivatizing agent with pyridine utilized as a catalyst. O-Acetylation modification enhances the ability of LAS to eliminate hydroxyl radicals and

hydrogen peroxide and improve the derivatized LAS's antioxidant capacity (Luna et al., 2018). Similar results have been reported regarding carboxymethylated LAS. The carboxymethylated LAS demonstrated a higher antioxidant potential than the unmodified LAS (Theis et al., 2017). The phosphorylation of LAS was explained by Sechi et al. (Sechi, 2017), in which LAS was phosphorylated with sodium trimetaphosphate resulting in a derivative with a low degree of substitution (DS, 0.014). Phosphorylation promoted a significant increase in the solubility (52.4%) of LAS in water (Sechi, 2017). The sulfation of LAS produced by *L. theobromae* strain MMLR was reported by Vasconcelos et al. (Vasconcelos et al., 2013). In the study, formamide was employed as the solvent, with pyridine and chlorosulfonic acid employed as the catalyst, and sulfation agent respectively. Sulfation of LAS (DS, 0.95) enhanced the anticoagulant activity, in a dose-dependent manner (Vasconcelos et al., 2013). Also, Calegari et al. (Calegari et al., 2017) sulfated LAS extracted from a different strain of *L. theobromae* (MMPI) using a similar method to the method described by Vasconcelos et al. (Calegari et al., 2017). The modification enhanced the antioxidant activity of LAS (DS, 0.24), with emphasis on the hydroxyl radical removal capacity (74.32%). Another important observation was that sulfation enhanced antimicrobial activity of the sulfated LAS against Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella enterica Typhimurium* ATCC 0028) and yeasts (*Candida albicans* ATCC 118804 and *Candida tropicalis* ATCC 13803) (Calegari et al., 2017; Cohen & Merzendorfer, 2019).

4.3.2. Chitin and Chitosan

Chitin, chitosan, and their β-glucan complexes (i.e., chitosan–glucan complex (ChGC), chitin–glucan complex (CGC), etc) are major PSs obtained from the cell walls of numerous fungi, e.g., *Gongronella* spp., *Absidia* spp., *Aspergillus* spp., *Rhizopus* spp. (Araújo, Ferreira, Torres, Neves, & Freitas, 2020; Nwe, Furuike, & Tamura, 2011). Among them, *G. butleri* provided the maximum yield of chitosan (2 g/100 g mycelia) (Nwe, Furuike, & Tamura, 2009; Nwe, Stevens, Montet, Tokura, & Tamura, 2008).

Due to their outstanding biological properties such as non-toxicity, progressive biodegradability, biocompatibility, immunomodulatory, anticancer, antioxidant, and antimicrobial activities, fungal chitin and chitosan have been the focus of extensive research over the last decades and have a wide range of applications in various fields such as biomedical, food industry, and agriculture (Ahmad et al., 2020; Araújo et al., 2020; Elsoud & El Kady, 2019; Insuasti-Cruz et al., 2021; Jones, Kujundzic, John, & Bismarck, 2020; Tchemtchoua et al., 2011). Furthermore, because crustacean chitin and chitosan structure is inconsistent, fungal sources are a good alternative, especially for biomedical and pharmaceutical applications (Elsoud & El Kady, 2019). As a result, in recent years, biotechnological

production of chitin and chitosan from fungal sources has gained extensive worldwide attention over conventional production of chitin and chitosan from Crustacea shell waste such as shrimp, crab, prawn, and crayfish (Razak, Pinjari, Begum, & Viswanath, 2018) and in the near future, it is expected that the use of fungi as a source of chitinous polymers will increase (Araújo et al., 2020).

Tchemtchoua et al. used an Ultrapure, medical-grade fungal chitosan provided from Kitozyme (Belgium) and produced films, nanofibers, and sponges for use as wound dressings. Based on the results, the best performance in wound healing, especially for the treatment of deep ulcers, was achieved by the chitosan nanofibrous scaffold produced by electrospinning (Tchemtchoua et al., 2011). Also, fungal chitin-based polymers revealed the great potential to be used as biomaterials to fabricate hydrogels and nanoparticles as drug delivery agents (Freitas, Roca, & Reis, 2015). More studies regarding the biomedical applications of fungal chitin and chitosan are discussed in section 8.3.1.

4.3.3. Sacchachitin (SC)

SC is a water-insoluble PS extracted from *Ganoderma tsuga* and *Ganoderma lucidum* (Chuang et al., 2013). Commercialization of fungi-derived wound healing materials started in 1997 by extracting SC. The SC is composed of about 40% chitin and 60% β-1,3-glucan. An SC film comprised of 10∼50-μm fibers showed promising wound healing (Chao et al., 2020; Jones et al., 2020; Nawawi et al., 2019). The wound-healing effects of the chitin sheet from crab shell (Beschitin) and SC from *G. tsuga* were comparable (Chuang et al., 2013; Smelcerovic et al., 2008).

4.3.4. Pullulan (PUL)

PUL is made by several strains of *Aureobasidium pullulans* (Selvasekaran, Mahalakshmi, Angalene, Chandini, & Chidambaram, 2021) which contains α-(1, 6)-linked maltotriose units, as a distinctive linkage configuration is considered to be responsible for the structural flexibility and solubility of PUL, leading to the unique film- and fiber-forming characteristics (Leathers, 2003). PUL is water-soluble and has wound healing and antibacterial activities (Kofuji et al., 2010; Ram S. Singh, Saini, & Kennedy, 2008). It is instantly biodegradable and is greatly resistant to temperature (its decomposition happens above 200℃ with no discharge of toxic gases) (Verma, Kumar, Jeslin, & Dubey, 2020).

There are other sources of fungal EPSs such as marine and endophytic fungi with potential applications in the biomedical area that remain still largely unexplored and unexploited and there are not many studies concerning their biological activity (Corinaldesi, Barone, Marcellini, Dell'Anno, & Danovaro, 2017; H. Li, Huang, Zhang, &

Yan, 2020; Orlandelli, Vasconcelos, Azevedo, da Silva, & Pamphile, 2016). Table 1 highlights and summarizes some major EPSs that may be sourced from fungi.

5. Extraction methods of fungal EPSs

The extraction approach of fungal EPSs is a function of the source, structure, and required degree of purity (Elsehemy et al., 2020; Zhu, Du, & Xu, 2016). For example, lentinan (from common edible mushroom *Lentinus edodes*) is extracted by ethanol precipitation, solubilized by acetic acid followed by chromatographic column purification (Venkatachalam, Arumugam, & Doble, 2020).

Unlike cell walls or cytosolic PSs, EPSs do not need multiple and complex steps for extraction using toxic organic solvents like hexane, or high concentration alkali solutions (Osińska-Jaroszuk et al., 2015). The EPSs like LAS are soluble and secreted into the growth medium during Smf and are easily collected by precipitation with ethanol, making their separation easier and cheaper than extractive processes for glucans from fungal fruiting bodies or yeast cell walls (Kagimura et al., 2015). Crude fungal EPSs can be obtained as a vacuum-dried or lyophilized powder after dialysis for the solubilized EPS against water (Cohen & Merzendorfer, 2019; da Silva Fonseca et al., 2020; Kagimura et al., 2015). Ion exchange chromatography (IEC) and gel permeation chromatography (GPC) can be used to purify the EPSs (Osińska-Jaroszuk et al., 2015).

Table 1. Major exopolysaccharides (EPSs) may be sourced from fungi.

EPS	Source(s)	Types of Glycosidic linkages	Monosaccharide Constituents	Some notes	Ref.
Pullulan	*Aureobasidium pullulans, Pullularia pullulans*	α (1,6), α (1, 4)	D-glucose	It can be used to fabricate nanofibers/particles and flexible coating due to its mechanical strength, high solubility in water, and insoluble in organic solvents (even water-miscible solvents such as ethanol).	(Chemspider, 2021; Jiang, Singh, Choi, Akaike, & Cho, 2015; Subhadip Mahapatra & Debdulal Banerjee, 2013a; Prameela, Murali Mohan, & Ramakrishna, 2018; Ruiz-Herrera & Ortiz-Castellanos, 2019; Ram S. Singh et al., 2008)
Scleroglucan	*Sclerotium glucanicum, Sclerotium rolfsii*	β (1,3), β (1,6)	D-glucose	Stable over wide ranges of pH and temperature and so can be applied in dressings and ice creams. It can also be used in the manufacture of cosmetics i.e., conditioners, shaving foam, etc. It has also been recently suggested that the EPS may possess antiviral effects that may be applicable in managing the viral effects of COVID-19. The molecular weight is ~2000 kDa.	(Natalia A. Castillo, Valdez, & Fariña, 2015; Geller & Yan, 2020; Jindal & Singh Khattar, 2018; Kırtel, Avşar, Erkorkmaz, & Öner, 2017; Subhadip Mahapatra & Debdulal Banerjee, 2013a; *Microbial Polymers, Applications, and Ecological Perspectives*, 2021; PubChem, 2021b; J. Song et al., 2020; Valdez, Delgado, & Fariña, 2021)
Botryosphaeran	*Botryosphaeria rhodina*	β (1,3), β (1,6)	D-glucose	Strong anticlastogenic, hypoglycemic, and hypocholesterolemic effects with antioxidant and free-radical scavenging properties. Soluble in water and is also capable of stable gels formations. The molecular weight is 1,820 kDa.	(Barbosa, Steluti, Dekker, Cardoso, & Corradi da Silva, 2003; de Lourdes Corradi da Silva et al., 2005; Geraldelli et al., 2020; Giese et al., 2009; Subhadip Mahapatra & Debdulal Banerjee, 2013a; PubChem, 2021a; Ruiz-Herrera & Ortiz-Castellanos, 2019; Selbmann, Stingele, & Petruccioli, 2003; Steluti et al., 2004; Weng et al., 2011)
Yeast β-glucan	*Saccharomyces cerevisiae*	β (1,3), β (1,6), α (1, 4), β (1, 4)	D-glucose	A fungal β-glucans with positive effects for the treatment of several diseases such as hypercholesterolemia and diabetes. The molecular weight ranges from 27.9kDa to 175 kDa.	(Bastos et al. 2022; CheBI, 2020; Du, Meenu, Liu, & Xu, 2019; Kwiatkowski & Kwiatkowski, 2012; Subhadip Mahapatra & Debdulal Banerjee, 2013a)
Schizophyllan	*Schizophyllum*	β (1,3), β (1,6)	D-glucose	A fungal β-glucans with positive effects	(M. C. Barcelos et al., 2020; CheBI, 2020;

	commune			for the treatment of several diseases such as hypercholesterolemia and diabetes. Can aid in reducing or preventing metastasis and lowers the side effects of chemotherapy. It is also employed in skincare products (as an anti-aging and healing agent), metal sorption from water, drug delivery, and in the fabrication of nanofibers. The molecular weight is 450 kDa.	Du et al., 2019; Subhadip Mahapatra & Debdulal Banerjee, 2013a; Sutivisedsak, Leathers, Nunnally, Price, & Biresaw, 2013)
Pestan	*Pestalotiopsis sp.*	β (1,3), β (1,6)	-	Can be used for biosorption of toxic metallic ions i.e., Cu^{2+}. The EPS has a molecular weight of 329.4 kDa.	(Subhadip Mahapatra & Debdulal Banerjee, 2013a; Osińska-Jaroszuk, Sulej, Jaszek, & Jaroszuk-Ściseł, 2020) (Mezcua, Malato, Garcia-Reyes, Molina-Diaz, & Fernandez-Alba, 2009)
Lentinan	*Lentinula edodes*	β (1,3), β (1,6)	D-glucose	Immunomodulating glucan has been used to treat patients suffering from gastric cancers, malignant effusions, and management of patients with the human immunodeficiency virus. Useful after cardiopulmonary bypass via ameliorating the impairment of natural killer cell activity. It is widely used as a hypocholesterolemic agent.	(Aronson, 2015; Mohd Jamil et al., 2013; D. Yang, Zhou, & Zhang, 2019; Y. Zhang et al., 2018)
Auricularian	*Auricularia polytricha*	α (1,4), α (1,3), β (1,3)	D-glucose	The β-glucan-containing EPS of auricularian has been reported to exhibit immunomodulatory activity against *Cryptococcus neoformans*. The possibility of its use in cancer treatment was also demonstrated in a previous study with auricularian shown to increase cancer survival rates by significantly 0.5 -2 years depending on individual case severity. The EPS has a molecular weight of 55.9 kDa.	(Miao et al., 2020; G. Song & Du, 2010, 2012) (D. Yang et al., 2019)

Elsinan	*Elsinoe leucospila* and other *Elsinoe* species	α (1,3), α (1,4)	D-glucose	Elsinan shows a huge dietary fiber impact and subsequently diminishes the cholesterol level in the serum of hypercholesterolemic individuals. This EPS can be shaped into several forms, and these forms are edible, nontoxic, transparent, hot water soluble, moisture, water-resistant, and extended timeframe without losing their appealing properties. Elsinan has a molecular weight ranging from 600-700 kDa.	(Selvasekaran et al., 2021; Synytsya & Novak, 2014)
Galactomannan	*Aspergillus* sp. and *Penicillium* sp.	β (1,4), α (1,6)	D-galactose and D-mannan	The EPS may be used in drug release due to its ability to resist bacterial degradation. Other properties such as hydrophilic nature, and nonionic nature, promote its use as a film-forming agent, coating agent, gelling agent, stabilizer, thickener, and cometic material. The EPS has a molecular weight of ~ 2560 kDa.	(Albuquerque, Coelho, Correia, Teixeira, & Carneiro-da-Cunha, 2016; Selvasekaran et al., 2021)
Pleuran	*Pleurotus ostreatus*	β (1,3), β (1,6)	D-glucose	This EPS has been reported to present immunomodulatory activity via the use of biological response modifiers. It is also capable of modulating the blood-producing activity of bone marrow. More work is, however, required to explore further the full range of biomedical applications of the Pleuran. The EPS has a molecular weight ranging from 100-10,000 kDa.	(Maftoun, Malek, Abdel-Sadek, Aziz, & Enshasy, 2013)

6. Necessity of optimization for fungal EPSs production

The yield of fungal EPSs may vary widely and depend on several environmental factors, physical conditions, and fermentation methods. For instance, most EPS-producing fungi are aerobic or facultative anaerobic, implying that the absence of oxygen reduces the yield of EPSs (Subhadip Mahapatra & Debdulal Banerjee, 2013b). Furthermore, process conditions such as substrate concentrations may influence EPSs yield (Joshi, Patel, Gupte, & Gupte, 2013). For instance, Joshi et al. explored the effect of process parameters (concentration of carbon and nitrogen sources of xylose (2.5-4.1 g % w/v) and yeast extract (0.83- 1.37 g % w/v), respectively and KCl (6.61-8.39 mg %w/v)) for the enhanced production of SPG by *S. commune*. The study determined that the optimum SPG yield of 4.26 g/L was generated by *S. commune* AGMJ-1 at the xylose, yeast extract, and KCl concentrations of 2.5 g % (w/v), 0.83 g % (w/v), and 6.53 mg % (w/v), respectively (Joshi et al., 2013). Yoon et al., (Yoon et al., 2012) also investigated the optimal low-cost production of EPSs from *Aureobasidium pullulans*' fungi via determining the preferred medium composition using Plackett–Burman and Box-Behnken design. The study showed that a 24-fold increase in EPS production in the optimized media was obtained relative to the concentration of EPS (1.2 g/L) produced in the reference basal medium (Yoon et al., 2012).

7. Modification of EPSs composition

Chemical modification of fungal EPSs (Table 2) is beneficial for biomedical applications. The chemical modifications such as oxidation, sulfation, and succinylation allow particular properties of various polymers to be combined, like most PSs, EPSs contain–OH groups prone to chemical and ionic modifications (Dionísio et al., 2016). It is possible to create new EPSs derivatives with regulated sequences and structures using contemporary chemical, biological, and analytical methods. Additionally, some functional groups such as carboxymethyl, acetate, phosphate, sulfate, and alkyl esters have been exposed to modify the composition of EPSs and meet the specific needs of the intended applications (Table 3 and Fig. 3).

Table 2. Some examples of chemical modification in fungal EPSs highlight the properties and applications of the modified EPS.

Polymer	Modification	Method	Properties and application	Ref.
Pullulan	**Sulfation**	Sulfation of pullulan using chlorosulfonic acid. Alternatively, using sodium bisulfite-sodium nitrite reagent system.	Sulfated pullulan has an important place in medicine and biology due to their ability to act as blood-compatible anticoagulants and used for selective adsorption of low-density lipoprotein.	(Alban, Schauerte, & Franz, 2002)
	Periodate oxidation	Sodium periodate was used for oxidation of an α (1,4)-linked anhdroglucoside unit in pullulan.	The periodate-treated pullulans are much more sensitive to acid hydrolysis than the intact samples.	(Bruneel & Schacht, 1993a)
	Succinylation	The succinylation of pullulan by reaction with succinic anhydride in dimethylsulfoxide as solvent and *N, N′*-dimethylaminopyridine as a catalyst. The highest reactivity of the hydroxyl group of pullulan to phenyl isocyanate in DMSO was established for the hydroxyl group at the C-6 position of the D-glucopyranose ring.	This derivatization has been a promising polymeric carrier for many drugs since the introduction of negative charges into the macromolecules.	(Shingel, 2004)
	Chloroformate activation	These derivatives of pullulan were prepared by reacting the parent polymer with varying amounts of chloroformate.	The 4-nitrophenyl chloroformate activation of pullulan is an easy method for obtaining amine-containing pullulan derivatives.	(Bruneel & Schacht, 1993b)
Botryosphaeran	**Sulfonation**	Botryosphaeran was sulfonated using pyridine and chlorosulfonic acid in formamide.	Botryosphaeran was derivatized by sulfonation to induce anticoagulant activity.	(Mendes et al., 2009)
Lentinan	**Sulfation**	Sulfation modification of lentinan was conducted with chlorosulfonic-pyridine and concentrated sulfuric acid methods.	All sulfated derivatives of lentinan show the higher reactivity of the hydroxyls on the C6 position than those at other positions.	(Xue-qian et al., 2009)
Scleroglucan	**Sodium carboxymethylation**	Hydrophobic stearate and sodium carboxymethyl groups were grafted onto the hydroxyl functions of scleroglucan.	Carboxymethylated scleroglucan has amphiphilic properties and showed potential applications in the formulation as an	(Bakhshi, Ozeiri, Sharif, & Aalaie, 2017)

		enhanced oil recovery agent.	
Oxidation	Oxidized scleroglucan (Sclerox) fabricated through a two-step reaction, using, in sequence, the first periodate, to form an aldehydic derivative, and then chlorite, leading to the carboxylated derivative.	Sclerox becomes sensitive to environmental conditions giving a reversible sol-gel transition mediated by pH, commonly used for drug delivery.	(Tommasina Coviello et al., 2005)

Table 3. Functional groups naturally found on fungal EPSs (red √) and reported chemical backbone modification (blue √) (Alban, Schauerte, & Franz, 2002; Bakhshi, Ozeiri, Sharif, & Aalaie, 2017; Cohen & Merzendorfer, 2019).

EPS	OH(I)	OH(II)	COOH	NH_2	SH	O_3P	AcOH
Pullulan	√	√	√	√	√		
Scleroglucan	√	√	√		√		
Schizophyllan	√	√	√				
Auricularian	√	√	√		√		
Lasiodiplodan	√	√	√		√	√	√

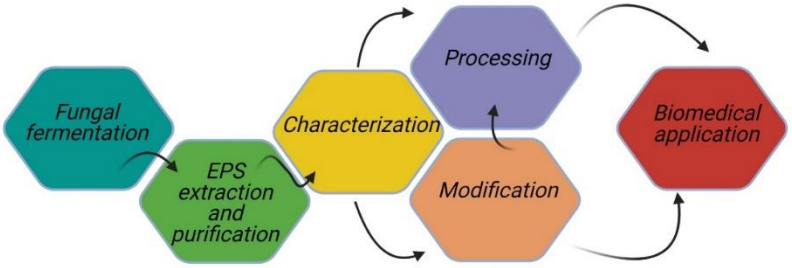


Fig. 3. The proposed life cycle of using fungal EPSs for biomedical application.

For example, oxidation of the pyranose ring in EPSs rewards more versatility in the configuration of the EPSs. For instance, oxidized derivatives of Scl (Tommasina Coviello et al., 2005) and PUL (Alban et al., 2002) have been investigated and shown to produce a reversible sol-gel transition. Ionizable functional groups, including carboxylate and sulfate, provide developing polymer solutions with various degrees of viscosity (Laurienzo, 2010; Tiwari, Patil, Dubey, & Bahadur, 2019). Negatively charged PUL might behave as a blood coagulant after anionic

alteration (by sulfation) (Hezarkhani & Yilmaz, 2019). The anticoagulant activity of the new PUL (sulfated PUL) has been reported was nearly the same as heparin. Still, the activity profile of the sulfated PUL depended on the degree of substitution, MW of PUL, and distribution of the sulfated groups on the numerous positions of the glucose monomers (Alban et al., 2002).

8. Application of fungal EPSs in tissue engineering, drug, and gene delivery

Fungal EPSs have several applications in the medicine, cosmetic, food, and pharmaceutical industries (Schmid et al., 2016). Indeed, the most common use of fungal EPSs is in medicine (Giavasis, 2014). In recent years, many reports regarding various biomedical applications of fungal EPSs, especially in TE, drug, and gene delivery, have been reported. Table 4 summarizes some studies using fungal EPSs for different biomedical applications, especially TE and drug delivery.

Table 4. Different biomedical applications of some commonly used fungal EPSs.

EPS type	Additional substant	Chemical Modification /cross-linking	Construct form and its application	Study design	Ref.
β-Glucan (βG) (β-(1,3–1,6)-D-Glucan isolated from black yeast (*Aureobasidium pullulans*))	Commercial chitosan	No	A transparent wound dressing sheet for incisional full-thickness wounds. 1.0% βG–1.0% chitosan complex sheet accelerated wound healing following 14-days post-treatment.	1 cm in diameter circular wound created on the dorsal skin of each male ddY mice.	(Kofuji et al., 2010)
(β-(1,3–1,6)-D-Glucan isolated from (*A. pullulans*))	Gelatin	Cross-linked using 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride.	Fibroblast and keratinocyte cells were cultured on a porous sponge scaffold as an artificial dermis.	A full-thickness skin defect on mouse skin healed after one week with artificial skin rather than the acellular scaffold.	(S. B. Lee et al., 2003)
Pullulan isolated from *A. pullulans*)	Dextran (Mw 500 kDa.) and gelatin	Using chemical cross-linker, trisodium trimetaphosphate (STMP) at concentrations of 4, 8, 12, and 16 wt% 10 wt% and adding NaOH aqueous solution to provide an alkaline condition to activate cross-linking.	Nanofibrous scaffold.	*In vitro*	(Shi, Le Visage, & Chew, 2011)
Pullulan	Chitosan and	The TA/CS/PL composite membranes were cured in	Composite nanofibers as a wound dressing with	*In vitro*	(F. Xu, Weng,

	Tannic acid	an oven at 150℃ for at least 1 h to induce chemical crosslinking with citric acid.	antibacterial capacity.		Gilkerson, Materon, & Lozano, 2015)
Pullulan (molecular weight 200 KDa)	Collagen from rat tail	Sodium trimetaphosphate (STMP) was used as a crosslinking agent.	5% collagen–PUL was prepared and considered as a structured delivery template for cells and biomolecules in regenerative skin applications.	The hydrogel was implanted on excisional wounds in male C57BL mice.	(Wong et al., 2011)
					(Colinet, Picton, Muller, & Le Cerf, 2007)
Scleroglucan with Mw=1.4×10^6 Da.	Dextran methacrylate	The functionalization of Scl with carboxymethyl groups.	Injectable and in situ cross-linkable systems. Suitable for drug delivery and biomedical applications.	*In vitro*	(Corrente et al., 2013)
Scl with Mw=1.4×10^6 Da.		Scl was modified into its carboxymethyl derivate (Scl-CM300).	Drug delivery (the release of fluconazole, diclofenac, and betamethasone has been tested).	*In-vivo* experiments on rabbits evidenced optimal skin tolerability of Scl-CM300 hydrogels after topical application.	(Tommasina Coviello et al., 1999; Paolicelli et al., 2017)

8.1. Tissue engineering (TE)

Fungal EPSs have been used as temporary scaffolding, enabling transplanted cells to adhere, grow, and develop distinct roles. Different kinds of fungal EPSs, e.g., Scl, PUL, SPG, etc., have potential capabilities to be deployed as hydrogels for TE applications (Table 5). The high capacity of the EPSs to capture and release its cargo, such as drugs and proteins, is one of the essential EPSs applications in EPSs-based hydrogels.

Table 5. Some studies on the wound healing effects of fungal EPSs.

ESP type(s)	Source(s)	In vitro/cell type	In vivo	Remarks/main results	Ref.
β-Glucan (βG)	*Saccharomyces cerevisiae*	-	A randomized, double-blind, placebo-controlled phase II study	Local treatment of diabetic lower extremity ulcers with this βG shows good safety results. The EPS showed promising potential as a treatment accelerating cutaneous healing. For example, in one patient who had an ulcer that would not heal for over 15 years, this treatment made a 67.8% reduction in the ulcer.	(Zykova et al., 2014)
βG	*S. cerevisiae*	-	Venous ulcer healing in humans	The (1→3)-βG enhanced ulcer healing and increased epithelial hyperplasia, besides intensified inflammatory cells, angiogenesis, and fibroblast proliferation.	(Medeiros et al., 2012)
3 EPS extracts	*Akanthomyces pistillariiformis* BCC2694, *Cordyceps dipterigena* BCC2073, and *Phytocordyceps* sp. BCC2744	Normal human dermal fibroblasts (NHF) cells	-	The results revealed that the EPSs were biocompatible and inducers of high levels of IL-8 in NHF cells.	(Madla, Methacanon, Prasitsil, & Kirtikara, 2005)
An EPS extract	*Rhodotorula mucilaginosa* sp. GUMS16	-	Full-thickness wounds in the rats	*In vivo* embedding of nanofiber webs, including 2% of the EPS on the full-thickness wound, demonstrated a faster healing rate.	(Hivechi et al., 2021)
Schizophyllan (SPG)	*Schizophyllum commune*	Keratinocyte/ dermal fibroblast	-	The results showed that the SPG-based nanofibrous scaffolds could make improve cell adhesion. Also, SPG could increase cell proliferation and migration.	(Safaee-Ardakani et al., 2019)
βG–chitosan complex	β-Glucan isolated from black yeast (*Aureobasidium pullulans*)	-	Excision wound in mice	The complex sheet confirmed therapeutic effectiveness comparable or greater to that of Beschitin®W, a commercial wound dressing made from chitosan. Moreover, this product did not dissolve during the application time, did not stick to wounds, and was easily removable.	(Kofuji et al., 2010)
Pullulan (PUL) gel	*A. pullulans*	-	Excision wound model on rats	Histological assessment proved that the gel improved the wound re-epithelialization, dermal regeneration, blood vessels formation, and collagen synthesis than in control groups.	(Thangavel, Vilvanathan, Kuttalam, & Lonchin, 2020)

Polysaccharides-rich extract	*Ganoderma lucidum*		Streptozotocin-induced diabetic rats	Topical application of aqueous cream including 10% (w/w) of the PS extract was more effective than that with Intrasite gel in wound closure.	(Cheng et al., 2013)
βG	*S. cerevisiae*	-	Burn-induced oxidative tissue damage in the rat	βG treatments facilitated a reduction in malondialdehyde (MDA) and myeloperoxidase (MPO) levels, while also increasing glutathione (GSH) levels.	(Toklu et al., 2006)
βG	*S. cerevisiae*	-	Male diabetic db/db mice	All nanofiber treatments offered improved wound healing as compared to the negative control (water). All βG-nanofiber treated groups showed significantly enhanced wound healing as compared to the NoβG-nanofiber treated group.	(Grip et al., 2018)
βG	A mushroom strain fermentation	-	Full-thickness wounds in the rats	Hemocompatibility assay results indicated no significant influence on the activated partial thromboplastin time (APTT) and thrombin time (TT) and minor adsorption of human serum albumin (HSA). Regarding the wound healing of rat skin, the healing time was reduced by 48% using PVA/glucan film compared to cotton gauze.	(Huang & Yang, 2008)
βG	*Piptopor betulinus* fruiting bodies	Colon carcinoma cell line (Caco-2) cells (using in vitro scratch assay)	-	The βG revealed no toxicity on Caco-2 cells up to 1000 μgmL^{-1} and promoted cell migration on in vitro scratch assay, indicating a potential wound healing ability.	(de Jesus et al., 2018)

8.1.1. β-glucans (βGs)

βG wound dressings are wound healing agents characterized by wound proteases resistance properties (J. Majtan & M. Jesenak, 2018; Juraj Majtan & Milos Jesenak, 2018; Przybylska-Diaz, Schmidt, Vera-Jimenez, Steinhagen, & Nielsen, 2013; Sharifi-Rad et al., 2020). βGs improve wound repair by promoting macrophages infiltration, which motivates tissue granulation, types I and III collagen, and re-epithelialization. A commercial product called "soluble βG (SBG) gel" comprising βGs and methylcellulose was applied as a hydrogel for diabetic foot ulcers (DFUs)

(Cutting, 2017). The SBG 2.5% (w/v) used in the formula, is a β-1,3/1,6 glucan separated from the cell walls of *S. cerevisiae* (Cutting, 2017). The SBG shapes a gel at room temperature and has been verified to keep its immune-stimulating activity, mainly producing significant amounts of IL-8 and monocyte tissue factor (TF) (Engstad, Engstad, Olsen, & Østerud, 2002; Grip et al., 2018). Commercial dietary supplements originating from different fungal βGs in powdered extracts, tablets, capsules, teas, and syrups are on the market, e.g., Imunoglukan P4H® from *Pleurotus ostreatus* and LentinanXP in USA/Lentinex® in Europe from *Lentinula edodes* (Bulam, Üstün, & Pekşen, 2018).

β-1,3-glucan have been also used in several TE applications, such as bone scaffolds composed of chitosan/β-1,3-glucan/calcium phosphate ceramic (Belcarz et al., 2013; Borkowski et al., 2015; A Przekora & Ginalska, 2015; Agata Przekora, Palka, & Ginalska, 2014) and wound dressing nanofiber scaffolds based on β-1,3-glucan/polyvinyl alcohol (PVA) (Basha, Sampath Kumar, & Doble, 2017). Borkowski et al. (Borkowski et al., 2015) fabricated carbonated hydroxyapatite (CHAp)/β-glucan composite as the bone substitute and evaluated the healing efficacy in drilled bone voids model induced in the proximal tibial metaphysis of rabbits (Fig. 4A). They assessed the bone regeneration process using radiological images and histopathological analysis (Fig. 4B & C) and observed osteointegration of the implanted bone substitute tissue with no signs of graft rejection. Przekora et al. (A Przekora & Ginalska, 2015) evaluated the effect of osteoblastic cell differentiation on the synthesized chitosan/β-1,3-glucan/HAp composite and proposed the structure as the bone tissue engineering (BTE) scaffold. The fabricated composite promoted bone alkaline phosphatase activity, synthesis of bone ECM (type I collagen and osteocalcin), and synthesis of the mineralized nodule. The results confirmed the osteoconductive and osteoinductive potential of the prepared composite, which can be applied as the bone regenerating construct.

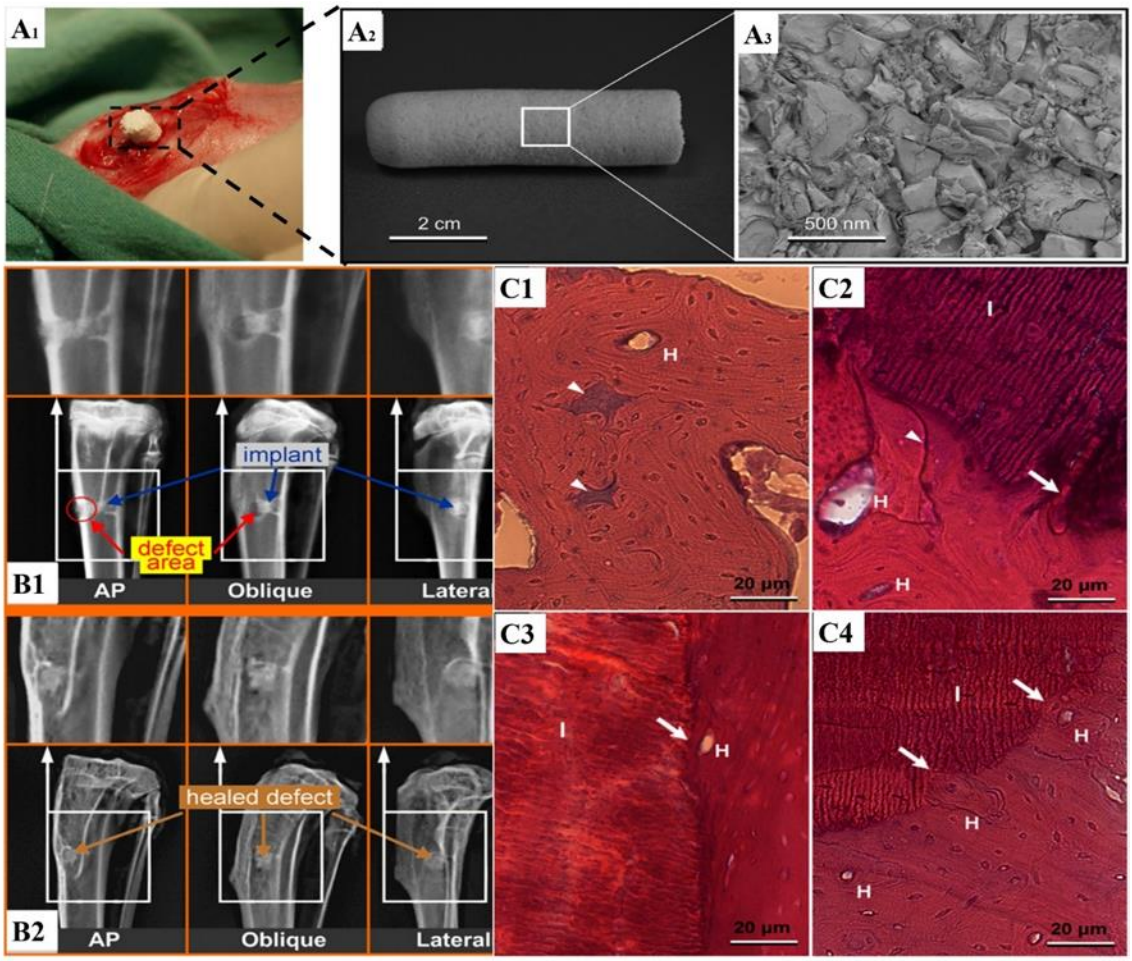


Fig. 4. (**A1**) Implantation of CHAp/glucan composite, (**A2**) Macroscopic image and (**A3**) SEM image of CHAp/glucan composite bone filler, (**B1**) X-ray (anteroposterior (**AP**), lateral and oblique) images of defect implanted with the CHAp/glucan composite at one month and (**B2**) six months, (**C1**) Histological images of a cross-section of the diaphysis in the metaphyseal proximal tibia in control rabbits and (**C2**) composite-implanted rabbits at 1 month, (**C3**) three months, and (**C4**) 6 months after implantation. Reprinted with permission from Ref. (Borkowski et al., 2015)

8.1.2. Scleroglucan (Scl)

Scl makes permanent gels in the presence of chromium salts and borax and can be precipitated by the addition of quaternary ammonium salts (Survase et al., 2007). Pseudoplasticity, or shear thinning, is the noticeable feature of Scl solutions (Survase et al., 2007). It was reported that amongst biopolymers, Scl and its derivatives emerge to be sufficient for the formulation of hydrogel matrices for steady drug release (Lapasin, Abrami, Grassi, &

Sebenik, 2017; Paolicelli et al., 2017); so, its applications for making hydrogel to use in TE and topical applications are achievable.

In a recent study, Bozoğlan et al. prepared some novel thermosensitive chitosan/carboxymethylcellulose/Scl/montmorillonite (CHT/CMC/SGL/MMT) nanocomposite hydrogels for potential applications in drug delivery, wound dressing, and TE (Bozoğlan, Duman, & Tunç, 2020). They found that gelling temperature of the hydrogels was near to the body temperature and the gelling temperature of the hydrogels was significantly influenced by MMT concentration (Bozoğlan et al., 2020).

8.1.3. Chitin and Chitosan

Chitinous polymers have been described to have significant antimicrobial activity against some fungi and bacteria (Nwe et al., 2011). Several studies (Feng et al., 2009; Hsieh et al., 2007; Lertwattanaseri, Ichikawa, Mizoguchi, Tanaka, & Chirachanchai, 2009; Ma, Wang, He, & Chen, 2001; Nie, Chen, et al., 2020; Nie, Deng, et al., 2020; Shanmugasundaram et al., 2001; Wan, Yu, Wu, Wang, & Wen, 2005; A. Wang et al., 2006) utilized chitosan isolated from shells of shrimps and crabs, and squid bone plates to make scaffolds for TE and examined the mechanical and biological characters of the scaffolds (Nwe et al., 2009); also chitinous polymers have been described to support the adhesion of nerve cells and neurite outgrowth, creating chitinous polymers as potential applicants for matrices in neural TE (Araújo et al., 2020; Smelcerovic et al., 2008). The chitinous polymers have been effectively utilized as wound-dressing ingredients and regulated drug release in several types, e.g., filaments, membranes, fibers, sponges, or composite with cotton or polyester (Araújo et al., 2020; Smelcerovic et al., 2008). For example, Chung et al. studied the potential of chitin/chitosan extracted from *Aspergillus oryzae*, *Mucor mucedo,* and *Phycomyces blakesleeanus* on the human fibroblasts proliferation rate. All the substances improved cell proliferation. Additionally, as *P. blakesleeanus* sample, which had the highest chitin content (91%), showed better proliferation activity than that of *A. oryzae* with a chitin content of 37%, indicating that the proliferative impact could be associated with their chitin quantity (Chung et al., 1994). Also, Mei-Yin Chien et al. showed that a chitin-containing mycelial mattress of *Rhizopus stolonifera* (called Rhizochitin) can be used for wound dressing (Chien et al., 2015). Rhizochitin had its beneficial role in wound healing by decreasing the expression of platelet-derived growth factor (PDGF) in the proliferation stage, raising the expression of transforming growth factor-beta (TGF-β) in the inflammation and proliferation stages, and intensifying vascular endothelial growth factor (VEGF) expression in the inflammation and proliferation stages. The in vivo studies also confirmed the wound healing efficacy of the mycelial

mattress (Fig. 5) (Chien et al., 2015) indicated by the epidermal layer formation and resemblance of the healed wound to normal skin.

8.1.4. Sacchachitin (SC)

It has been reported that SC and its derivatives have various biological activities beneficial for TE applications. Different studies evaluated the potential of SC for the TE constructs. Hung et al. have shown that SC membrane developed from the residue of the fruiting body of *G. tsugae* induced the same wound healing effects as BESCHITIN®, a chitin-based artificial skin. They also observed that the SC induced a chemotactic effect on the inflammatory cells and accelerated the acute inflammatory reaction while shortening the inflammatory period. They concluded that these phenomena may induce earlier tissue formation, beneficial for faster wound healing (Hung et al., 2001). Wu et al. also developed a one-pot fabrication of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)-oxidized SC nanofibers as the BTE scaffold. They utilized KOH and $NaClO_2$ as the alkaline agent in depigmentation and as the bleaching agent, respectively, in one pot. They reported that the synthesized TEMPO-oxidized SC nanofibers form a 3D gelatinous scaffold with excellent calcium-trapping ability due to the presence of a great extent of carboxylate groups. This may be due to the surface chemical modification of SC that provides carboxylate groups at the C6 position of the PS, which, when complex with calcium ions, may encourage bone regeneration. The in vivo studies revealed that the implantation of the TEMPO-oxidized SC nanofibers-based hydrogel induced good bone regeneration in a femur defect rat model (Wu et al., 2021). In the same approach, Chao et al. applied TEMPO-oxidized SC nanofibers-based hydrogel as the diabetic wound healing biomaterial. The morphological evaluation revealed the porous structure of the synthesized scaffold. The in vitro and in vivo studies showed that the fabricated hydrogels were biocompatible and accelerated diabetic wound healing process at similar rates to normal tissues and induced the growth of sweat glands and hair follicles (Fig. 5) (Chao et al., 2020).

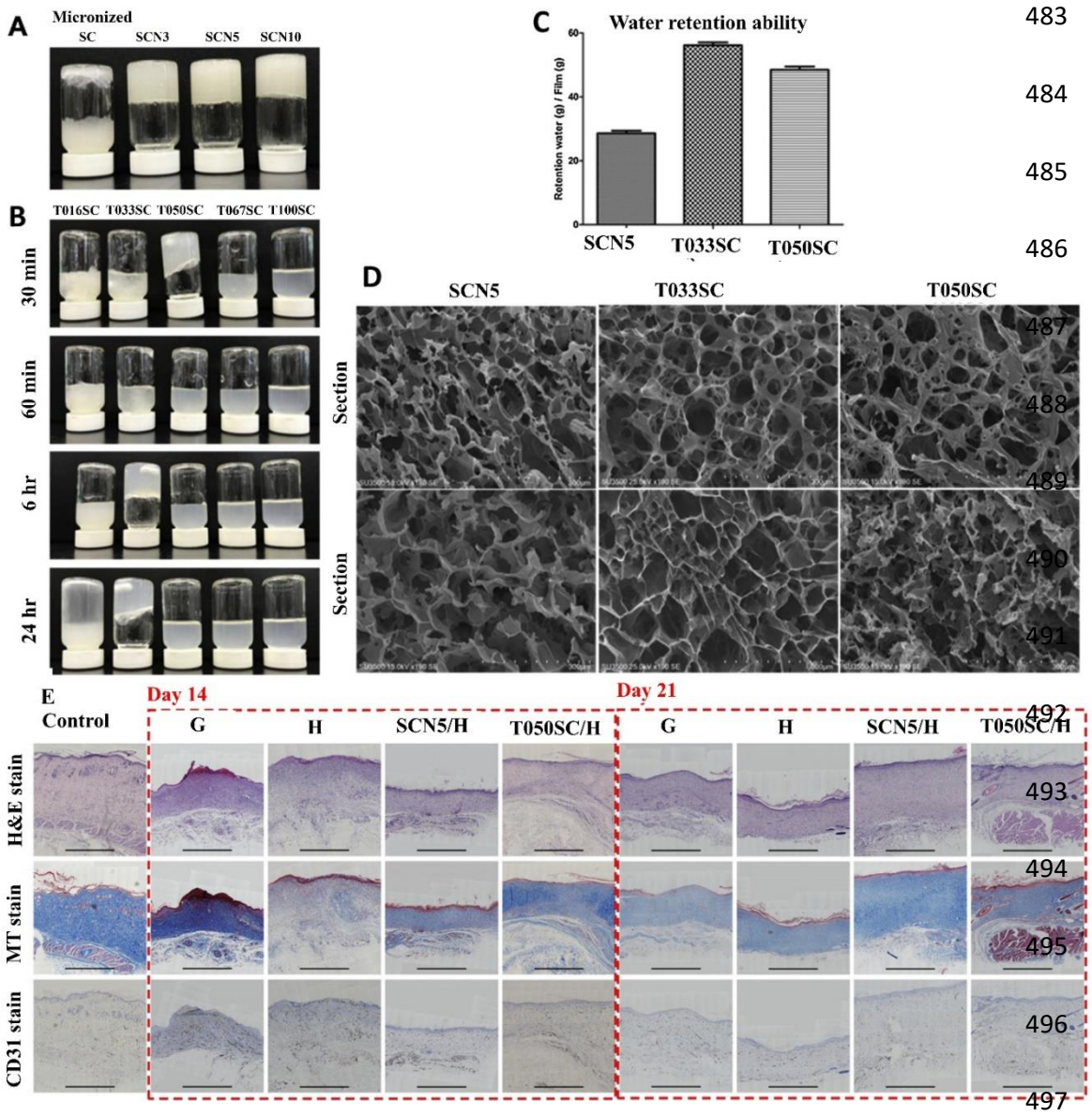


Fig. 5. Physical and biological properties of the fabricated SC nanofibers (**A**) and (**B**) Gel-forming properties, (**C**) The water-retention ability, (**D**) SEM images of the prepared hydrogels, (**E**) Histological analysis of the wound treated with the hydrogels. SC: Sacchachitin, SCN: Sacchachitin nanofibers, SCN3: SC mechanically digested for three cycles, SCN5: SC mechanically digested for five cycles, SCN10: SC mechanically digested for then cycles, TOSCNF: TEMPO-oxidized SCNF, TEMPO: 2,2,6,6-tetramethylpiperidine-1-oxyl, G: Gauze+PU film, H: AMP-based hydrogel, SCN5/H: SCN5/AMP-based hydrogel, T050SC/H: T050SC/AMP-based hydrogel, Reproduced with permission from Ref. (Chao et al., 2020)

8.1.5. Pullulan (PUL)

Due to their fascinating chemical and physical properties, PUL and its derivatives have shown promising results in TE applications. The utilization of PUL and its derivatives have shown regenerative effects in bone, skin, and vasculature TE applications. Fricain et al. fabricated a 3D macroporous nanocomposite scaffold based on nano-hydroxyapatite/PUL/dextran as the BTE scaffold. They reported that the scaffold induced multicellular aggregates formation and early and late bone-specific markers expression. The animal studies also revealed the osteogenic potential of the scaffold (Fricain et al., 2013). Iswariya et al. applied collagen blended PUL hydrogel for skin TE. They reported that the fabricated hydrogel exhibited a swelling ratio of up to 320%, ideal for the wet wound healing hypothesis. They reported that the hydrogel absorbed the wound exudates and minimized the trauma by providing a moist wound healing environment (Iswariya, Bhanukeerthi, Velswamy, Uma, & Perumal, 2016). Bae et al. fabricated cell-laden microscale tissues using PUL methacrylate (PulMA) to encapsulate cells in 3D environments. They reported that the construct provided organized cells cluster with controlled size. These structures can be applied as the cell-responsive micro-tissue complex with the ability to adjust the size of cell organization (Bae et al., 2011). In another attempt, Popescu et al. fabricated TE capsules based on alginate, PUL, and bioactive containing copper oxide. The in vitro results revealed that the capsules were biocompatible regarding the fibroblast and osteoblast and were osteoactive, investigated by soaking in simulated body fluid (SBF) solution. For the in vivo biocompatibility assessment, the capsules were implanted subcutaneously in Wistar rats, harvested after 5 weeks, and analyzed. The analysis showed that the capsules were biocompatible and tolerated by the host tissue (Popescu et al., 2018).

PUL has the potential to form injectable and in situ forming hydrogels appropriate for TE applications. Li et al. fabricated self-crosslinking and injectable based on PUL/chondroitin sulfate (CS) for cartilage TE. They functionalized CS with adipic dihydrazide (CS-ADH) and oxidized PUL (oxPUL) and fabricated hydrogel by covalent hydrazone crosslinking approach. They reported that the varying concentration of CS-ADH and oxPL adjust the gelation time, degradation behavior, mechanical properties, equilibrium swelling, and network morphology of hydrogels. They observed that the presence of CS in the structure favored cartilaginous ECM deposition and support rabbit articular chondrocytes viability, proliferation, chondrocyte phenotype, and enhanced chondrogenesis (T. Li et al., 2018).

8.1.6. Other fungal EPSs

In addition to the known EPSs extracted from different fungi, there are other EPSs that have not been classified in the above-mentioned categories and we reviewed these EPSs as the other fungal EPSs. Smirnou et al. extracted an EPS from *Cryptococcus laurentii*. They reported that changing pH from pH 3 to pH 6 increased glucuronic acid (GluAc) content, while decreasing galactose, xylose, and glucose content of EPS. They assessed the wound healing efficacy of the extracted EPS and observed EPS significantly improved excisional wound healing (Smirnou et al., 2014). In another study, Hivechi et al. extracted an EPS from *R. mucilaginosa* sp. GUMS16 and applied as the bioactive agent to polycaprolactone (PCL) and gelatin nanofibers. They reported that the produced EPS was a highly branched glucan. The authors applied nanofibers as the wound dressing material in the animal model and evaluated the healing efficacy using macroscopic observation by wound closure calculation and microscopic assessment using histopathological analysis (Fig. 6). As seen in Fig. 6Aa, the EPS includes peaks at 3375 cm^{-1}, 1647 cm^{-1}, 1414 cm^{-1}, and 1080 cm^{-1}. These peaks correspond to O-H stretch hydroxyl, C=O stretch, C-H bending (CH2), and S=O or C-O stretching functional groups.. The TGA plot (Fig. 6Ab) depicts a two-step degradation process that begins at roughly 280℃ and ends at 350℃, with weight losses of 20% and 65%, respectively. The ultimate residual mass of EPS is roughly 3%, showing that the majority of this material dissolves at 750℃. DSC data revealed two exothermic peaks at 280 and 390℃. This data shows that EPS degrades in two phases, each of which generates a large quantity of heat. Fig. 6Ac is an SEM picture of EPS particles. Data reveal that EPS particles have an average diameter of roughly 40 nm. DLS studies yielded a hydrodynamic diameter of 42.7 nm, which correlates with SEM observations. The morphology of the manufactured PCL/Gelatin (PCL/Gel) mix nanofibers encapsulated with 0–2 percent EPS is shown in Fig. 6B. For biomedical applications, they found that the nanofibers' diameter directly influences their characteristics, such as porosities and permeability as well as cell attachment and degradation rates. The incorporation of the EPS had a considerable influence on the distribution curves, according to the results. When EPS was added to samples the mean diameter of the nanofibers decreased from 161 nm to as little as 158 nm for the lower concentrations (EPS 1%) and as little as 144 nm for the higher concentrations (EPS 2%). The animal studies showed that incorporating the EPS improved the wound closure percent from 72.33 ± 2.1% for PCL/Gelatin nanofiber to 99.81 ± 1.39% for PCL/Gel/2% EPS. They proposed that the possible antioxidant activities of the produced EPS may accelerate the healing process (Hivechi et al., 2021) (Fig. 6C and D).

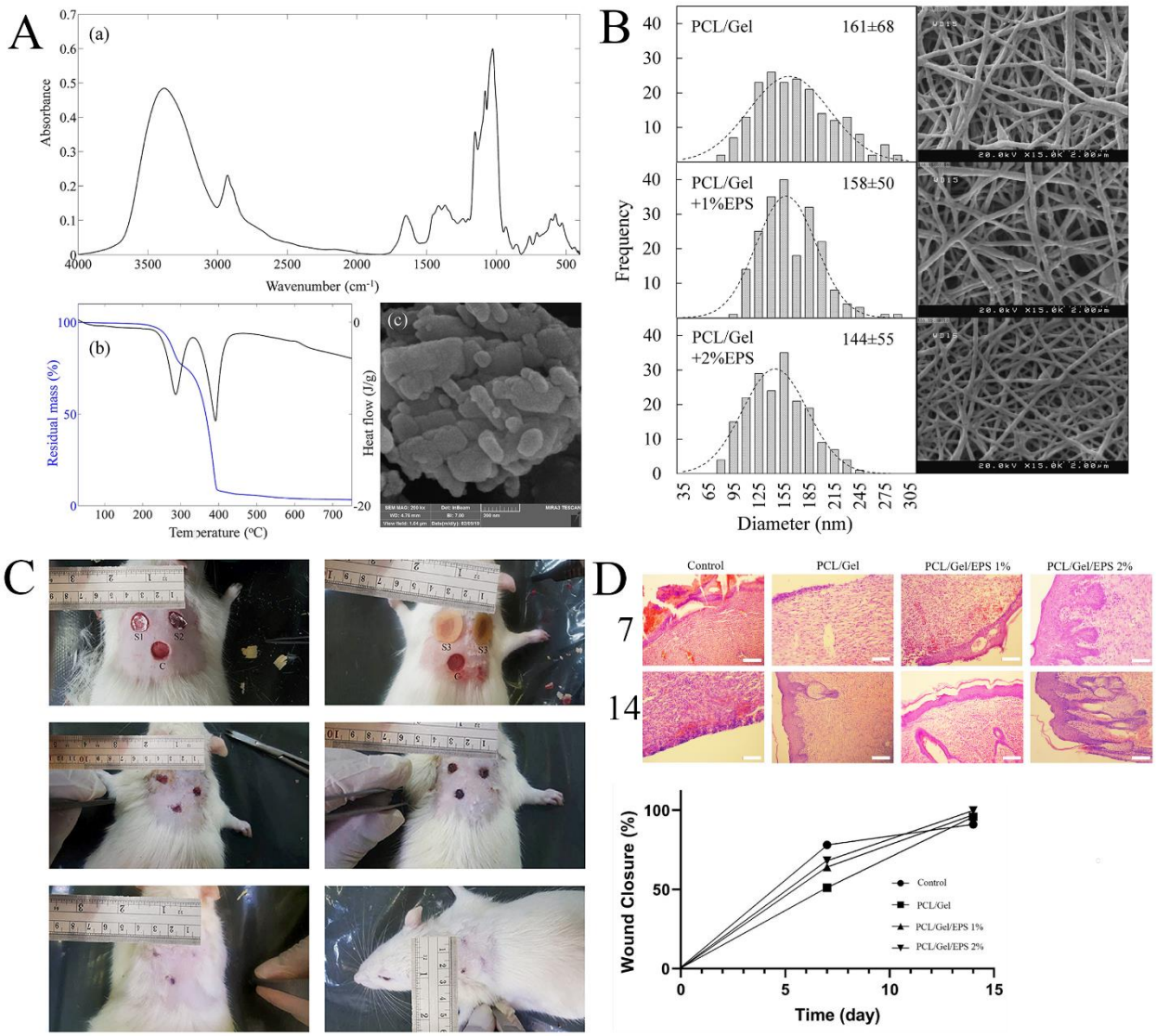


Fig. 6. The effects of nanofiber scaffold containing EPS on wound healing in rat model, **(A)** FTIR spectrum (**a**), TGA (blue)/DSC (black) diagram (b), and SEM images of the produced nanofiber containing the EPS (c), **(B)** SEM photographs (right) and fiber diameter distribution (left) of the nanofiber samples (PCL/Gel) with different EPS contents: (a) 0% EPS, (b) 1% EPS, and (c) 2% EPS, **(C)** Photomicrograph of wound closure during 14 days follow up, **(D)** Histopathology results of the treatment. Reproduced with modification from (Hivechi et al., 2021)

8.2. Drug delivery

8.2.1. Pullulan (PUL)

PUL and its derivatives are widely used as the drug delivery i.e., drug carrier, due to their high solubility, nontoxicity, structural flexibility, stability against digestive enzymes, and processability to formulate into microspheres, nanogels, hydrogels, or nanoparticles (Grigoras, 2019; Prajapati, Jani, & Khanda, 2013). Furthermore, due to the relatively high affinity of the lectin receptor in hepatocytes toward the sugar residues in the PUL structure, PUL can be considered as the promising targeted carrier for liver drug delivery applications (Tabernero & Cardea, 2020). There are nine hydroxyl groups in each repeating unit of PUL that can be substituted to produce various PUL derivatives and conjugate with various drugs (Ram Sarup Singh, Kaur, & Kennedy, 2015). For instance, PUL acetate (under reaction with acetic anhydride in the presence of formamide and pyridine), carboxymethyl PUL (under carboxymethylation reaction through interaction with isopropyl alcohol and sodium chloroacetate), PUL succinylated (under reaction with succinic anhydride), and PUL amine (through the chloroformate activation reaction) (Ram Sarup Singh et al., 2015). PUL acetate self-aggregates into a micelle-like structure with a hydrophobic core and hydrophilic shell able to encapsulate hydrophobic drugs (clonazepam, silymarin, etc.) and administrated for disease treatment, such as panic disorder, tumor, etc. (Young-II Jeong et al., 1999; Jung, Jeong, & Kim, 2003). Table 6 summarizes the studies conducted on PUL as the drug carrier.

Exosomes with cationic ethylenediamine-modified cholesteryl PUL (cCHP) nanogels and cationic ethylenediamine-modified cholesteryl PUL (cCHP) nanogels were recently introduced to be efficiently internalized into cells and enhanced functional transmission exosomes (Sawada et al., 2020). An amphiphilic PUL derivative, dibutylaminopropyl carbamate PUL octanoate (DBAP-PO) nanoparticle with drug-loaded showed sustained and pH-dependent release of a drug. Moreover, the nanoparticles had no cytotoxic effects at the pharmacologically relevant concentration of the drug (Constantin et al., 2020). A composite hydrogel composed of PUL hydrogel and polydopamine (PDA) fibers were developed through a one-step cross-linking strategy using poly (ethylene glycol) diglycidyl ether (PEGDGE) as the crosslinker. The content of PDA fibers significantly affects the mechanical and structural properties of the hydrogel. The addition and increasing the content of PDA fibers reduced the elastic modulus and the storage modulus of the structure since PDA contains phenolic hydroxyl groups and might impede the cross-linking reaction since its phenolic hydroxyl groups are relatively inactive and may not be able to interact

with the cross-linker. Furthermore, the H-bonds between PDA and PUL might avert the cross-linking reaction and the etherification process between PEGDGE and PUL (Su, Zhao, Wu, Dong, & Qi, 2020).

Synthesis of gold nanoparticles (AuNPs) using para-aminobenzoic acid-quat188-PUL (PABA-QP) as a trifunctional reducing/stabilizing/capping agent can form a nano vehicle to increase the anticancer activity of the drug (Laksee, Puthong, Kongkavitoon, Palaga, & Muangsin, 2018). The pH-sensitive folic acid (FA)-PABA-Q188-PUL@AuNPs enhanced intracellular drug uptake and showed high anticancer activity, revealed high anticancer activity, and less cytotoxicity toward body cells (Laksee et al., 2020). The spherical FA-PABA-Q188-PUL@AuNPs had high internalization through folate receptor-mediated endocytosis due to the relatively high affinity between the conjugated folate and folate receptors on cells, resulting in anti-tumoral effects (Laksee et al., 2020). It was reported that the conjugation of galactosylated PUL and curcumin resulted in an amphiphilic structure that easily formed micelle in an aqueous solution. The conjugation increased the solubility of curcumin and increased uptake and toxicity toward human hepatocellular carcinoma (HepG2) cells. Such conjugations are effective for target-specific delivery to hepatocarcinoma cells due to the relatively high affinity of the lectin receptor in hepatocytes toward the sugar residues in the PUL structure, which results in asialoglycoprotein mediated endocytosis of the formulation (Sarika, James, Nishna, Kumar, & Raj, 2015). In a similar approach, hydroxypropyl cyclosophoraose-PUL (HPCys-Pul) microspheres were formulated using the emulsion-crosslinking method. The microspheres exhibited efficient encapsulation of naproxen and maintenance of drug level in plasma after oral administration was longer (Choi et al., 2017).

It was reported that oxPUL can act as a “gatekeeper” in oxPL-coated-NH_2 grafted mesoporous silica nanoparticles (NH_2-MSN) which respond to acidic conditions and release the encapsulated 5-FU from the mesopores of MSN. The pH responsiveness is due to the hydrolysis of the acyl hydrazone bond between MSN and oxPL (S. Li et al., 2020). In another approach, hepatocellular carcinoma was targeted by PUL containing Dox through the programming of PUL. The programming involved the backbone oxidation of PUL and conjugation of Dox and targeting peptide (PreS1) via a releasable linker. The peptide conjugation was conducted using a 3.4 kDa PEG spacer to the aldehydes present along the oxPUL backbone following reductive amination. The Dox conjugation was performed through a hydrazone pH-sensitive. The results showed that the formulation exhibited high selectivity toward serpine B3 receptor overexpressing cells (HepG2/SERPINB3 cells) and induced two-fold increased anticancer activity (Balasso et al., 2017).

621 **Table 6.** Drug delivery systems based on pullulan and its derivatives.

Pullulan derivatives	Structure	Particle size (nm)	Drug Concentration (drug/pullulan mg/mg)	Target organ/cells	Model	Ref.
Cholesterol-bearing pullulan	Nanoparticles (NPs)	20-30	Insulin 2/10	Rat blood	*In vivo*	**(Akiyoshi et al., 1998)**
	Nanogels	20-30	β-amyloid (Aβ1–42)	Primary cortical neurons and N9 microglial cells	*In vitro*	**(Boridy, Takahashi, Akiyoshi, & Maysinger, 2009)**
	Nanogels	10-20	Erythropoietin	Sprague–Dawley rats	*In vivo*	**(Hirakura et al., 2010)**
	NPs	-	Docetaxel	Human lung cancer cells	*In vitro*	**(Satoh, Chen, Aoyama, Date, & Akiyoshi, 2008)**
	NPs	32.6	Fluorescein derivative 1.5/37.4	Rat liver- Hepatoma cell line (HepG2)	*In vivo* and *in vitro*	**(Taniguchi, Akiyoshi, Sunamoto, Suda, & Yamamoto, 1999)**
	NPs	90–168	Mitoxantrone 1/5–15	Heart, spleen, liver, kidney, and lung sections from ICR mice	*In vivo*	**(W. Yang, Wang, Ma, Li, & Huang, 2014)**
Pullulan acetate	NPs	>100	Silymarin 10/20–40	Zebrafish embryo	*In vivo*	**(Kumar, Kumar, Suguna, Sastry, & Mandal, 2012)**
	NPs	50-100	Epirubicin	Human throat epidermal carcinoma cell line	*In vitro*	**(H.-z. Zhang et al., 2009)**
	NPs	250-33	Epirubicin 5/50	Human throat epidermal carcinoma cell line	*In vitro*	**(H.-z. Zhang et al., 2010)**
	NPs	50-60	Adriamycin 10/50	Human breast tumor	*In vitro*	**(Na, Lee, & Bae, 2003)**
	NPs	100	Adriamycin 20/50	HepG2	*In vitro*	**(Na, Lee, Park, et al., 2003)**
	NPs	50-130	^{99m}Technetium	Tumor cells in male Balb/c mice	*In vivo*	**(K.-H. Park et al., 2007)**
Carboxymethyl pullulan	Conjugate	-	Doxorubicin 0.7–1.67/10	Rat tumor cells	*In vivo*	**(Nogusa, Yamamoto, et al., 2000)**
	NPs	<100	Doxorubicin 0.32/10	Mouse breast cancer cells	*In vitro*	**(Lu et al., 2009)**
	Conjugate	-	Doxorubicin 0.61–0.71/10	Rat liver	*In vitro*	**(Nogusa, Yano, Okuno, Hamana, & Inoue, 1995)**
	Conjugate	-	Doxorubicin 0.56–0.69/10	Tumor cells in Wistar rats	*In vivo*	**(Nogusa, Yano, et al., 2000)**
Diethylenetriamine pentaacetic acid pullulan	Conjugate	-	Immunosuppressant	Arthritis in Lewis rats	*In vivo*	**(Masuda et al., 2001)**
	Conjugate	-	Interferon (INF)-β	Human liver	*In vivo*	**(Suginoshita, Tabata, Moriyasu, Ikada, & Chiba, 2001)**
Pullulan succinylated	Microspheres	22×10^4	Lysozyme 100/1000	-	*In vitro*	**(Fundueanu, Constantin, & Ascenzi, 2008)**
All trans retinoic acid bearing pullulan	NPs	159	Doxorubicin 10/50	A2780 cell line (doxorubicin sensitive and resistant)	*In vitro*	**(F. Li et al., 2013)**
	Nanogels	-	Doxorubicin 20-250	Human cervical carcinoma cells	*In vitro*	**(J. Lee, Jeong, Seo, & Na, 2013)**

Nanogels	121–163	Doxorubicin 2/40	Hela cells	*In vitro*	**(Seo, Lee, Jung, & Na, 2012)**

8.2.2. Scleroglucan (Scl)

Scl and its derivatives have shown promising potential as the drug delivery system because of their rheological properties, resistance to temperature, hydrolysis, and electrolytes resistance (Tommasina Coviello et al., 2005). Various studies used Scl as the matrix to obtain a controlled drug release (Tommasina Coviello et al., 2005). Casadei et al. fabricated a hydrogel-based on Scl as a delivery system for Theophylline by the acylation of Scl with one ω-dicarboxylic acid containing from two to six methylene groups in the chain. The resulted hydrogels could be suitable for drug-controlled release (M. Casadei, Pitarresi, Benvenuti, & Giannuzzo, 2005). Corrente et al. synthesized a pH-sensitive drug delivery system based on carboxymethylated Scl (Scl-CM) hydrogels cross-linked by $CaCl_2$ solution. The results showed that the Scl-CM/$CaCl_2$ ratio determined the release profile and increasing the $CaCl_2$ concentration provides tight and strength hydrogel influencing the drug release kinetics, the slower the release using the higher $CaCl_2$ concentration (Corrente et al., 2009).

In another study, Corrente et al. showed that Scl-CM can be applied to fabricate pH-sensitive physical hydrogels as the drug delivery system. They observed that the sol-gel transition occurred even in the absence of salts at the high carboxylation degree due to the presence of a great number of hydrogen bonds in the structure. The prepared hydrogels were loaded with four different nonsteroidal anti-inflammatory drugs (NSAIDs) and exhibited pH responsiveness beneficial for oral drug delivery applications. The pH responsiveness was due to the completely undissociated carboxylic groups at the at acid pH, which prevent the swelling and drug release. On the other hand, the carboxylic groups dissociated slightly in basic pH and induced electrostatic repulsions among the chains and subsequently hydrogel swelling and drug release. They proposed that the fabricated drug delivery system can be applied as the oral administration of ulcerogenic doses of NSAIDs (Corrente et al., 2012). Paolicelli et al. fabricated Scl-CM with a high degree of carboxylate for topical delivery of fluconazole, betamethasone, and diclofenac in the absence of drug-hydrogel interactions; in the case of fluconazole and betamethasone, drug release followed the Fickian transport model, while the hydrogen bonding between diclofenac and hydrogel induced a non-Fickian two-phase transport model (Paolicelli et al., 2017). The concentration of cross-linker and cross-linker to polymer molar ratio affect the release kinetics of the loaded drug. Corrente et al. observed that the release rate of the NSAIDs

depends on the $CaCl_2$ concentration and Scl-CM/Ca^{2+} molar ratio (Corrente et al., 2009). Altering the salt quantity in samples with the same polymer concentration and/or changing the molar ratio between carboxylated repeating units of the polymer and Ca^{2+} in general, may readily modify the amount of released drug (M. A. Casadei, Matricardi, Fabrizi, Feeney, & Paolicelli, 2007). Furthermore, it was feasible to achieve a system with zero-order release kinetics by an appropriate combination of hydrogels formed using different salt concentrations (Corrente et al., 2009).

It is possible to synthesis injectable and in situ cross-linkable (ISCL) hydrogels using Scl. Corrente et al. fabricated ISCL hydrogels constructed from dextran methacrylate (DEX-MA) and Scl, in its native form and carboxymethyl form (Scl-CM). Rheological properties of two DEX500-MA/Scl-CM and DEX500-MA/Scl systems were investigated to evaluate their mechanical properties and find out that the combination of polymers resulted in favorable mechanical properties, beneficial for biomedical applications. Moreover, they observed that small drugs (theophylline) were released very fast from the system. In contrast, larger drugs (vitamin B12 and myoglobin) exhibited controlled release because of the entrapment efficacy, the welling state of the hydrogels, and the pore size of the matrix (Corrente, Amara, Pacelli, Paolicelli, & Casadei, 2013).

8.2.3. Schizophyllan (SPG)

SPG has promising properties, such as biocompatibility, in vivo stability, and processability, which has made it a suitable carrier for various drug delivery applications. Naeeni et al. encapsulated ellagic acid into the SPG NPs for treatment of breast cancer and proper encapsulation efficacy and drug loading. The synthesized nanoformulation effectively inhibited the growth of MCF-7cells (Pirzadeh-Naeeni, Mozdianfard, Shojaosadati, Khorasani, & Saleh, 2020). Negahban et al. synthesized self-assembled micelles based on stearic acid-modified SPG for efficient delivery of paclitaxel. They reported that the esterified SPG is easily self-assembled into nano micelles (size ranged from 156 to 175 nm) through the sonication. The nanomicelles exhibited 75% encapsulation efficiency and a sustained release profile over 144 h (Negahban, Shojaosadati, & Hamedi, 2021).

Kim et al. synthesized hybrid nanogels comprised of SPG-methacrylate and ovalbumin-conjugated hyaluronic acid-methacrylate (HAMA-OVA) for topical delivery applications (Fig. 7). They reported that sonication and filtration significantly reduced the particle size since breaks the aggregates and excluded the larger particles. They modified SPG with methacrylic anhydride and observed that the modification enhanced the cellular uptake of nanogels with dendritic cells (DCs; JAWSII) through the mannose receptor-mediated internalization. Moreover, the

incorporation of HAMA-OVA promoted the penetration of nanogel into the porcine stratum corneum layer and its deposition in the dermis. They reported that OVA was effectively delivered to JAWS II cells and induced the cells' maturation and upregulation of activation marker interleukin-6 (Hyunkyu Kim, Lee, & Ki, 2020).

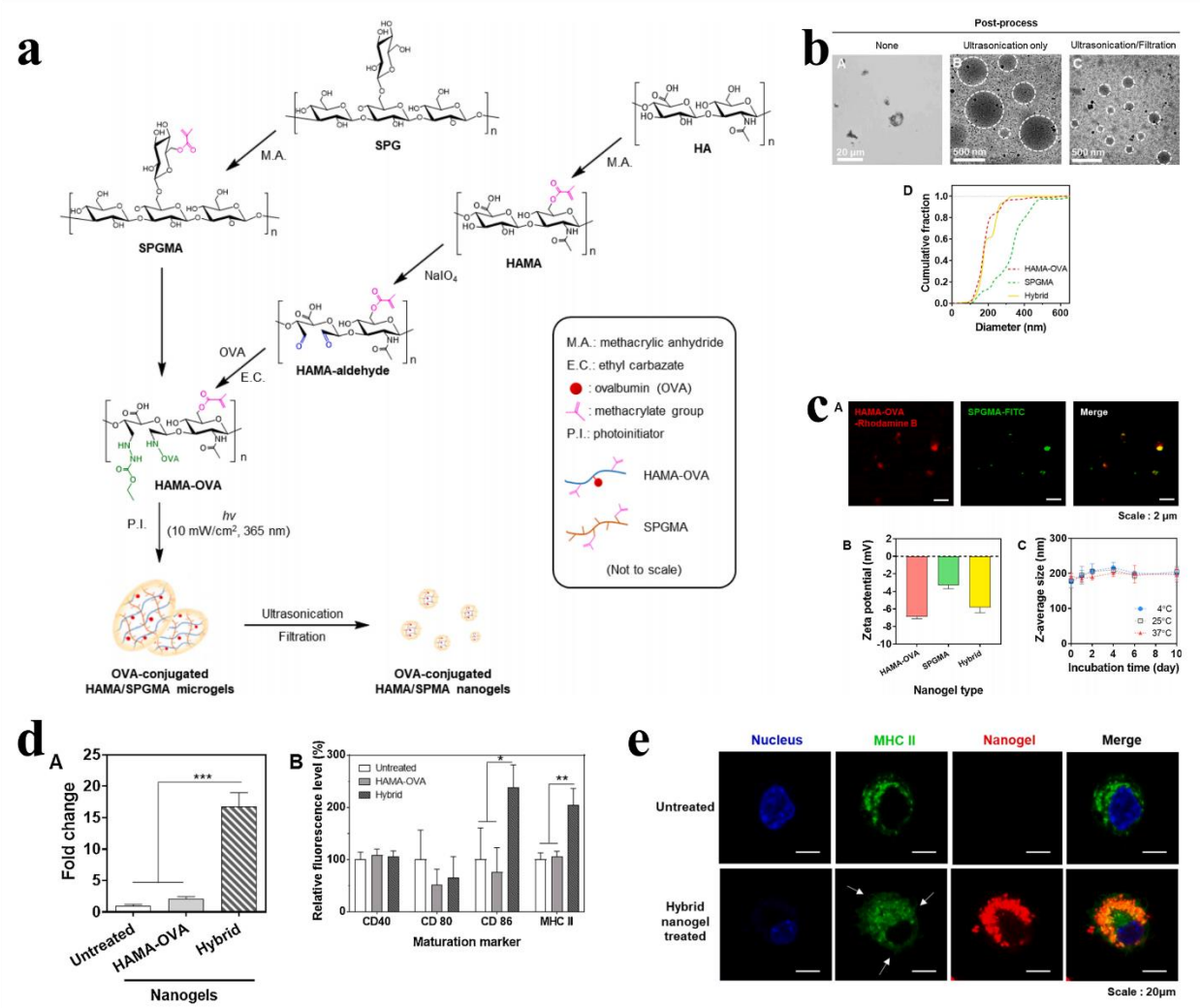


Fig. 7. (a) Schematic illustration of the nanogel synthesis. SPG methacrylated (SPGMA) through the reaction with methacrylic anhydride, using the same reaction, hyaluronic acid (HA) methacrylated using the same reaction (HAMA) and oxidized with sodium periodate to synthesis Ovalbumin-conjugated HAMA (HAMA-OVA) through the reaction between N-terminal amine of OVA and the aldehyde of HA. The nanogel formation was conducted through photocrosslinking during vortexing, followed by ultrasonication and filtration. **(b)** Morphology and size distribution of nanogels, **(c)** Properties of OVA-conjugated HAMA/SPGMA hybrid nanogels, **(d)** Nanogel induced

DC maturation marker and proinflammatory cytokine expression levels, **(e)** Confocal microscopy images of JAWS II cells treated with hybrid nanogels. Reproduced with permission from Ref. (H. Kim et al., 2020).

8.3. Gene delivery

8.3.1. Pullulan (PUL)

PUL and its derivatives have shown promising results as the carrier for gene therapy due to their fascinating properties such as low-cytotoxic, biodegradable, and non-immunogenic properties, as well as co-existence of α-(1 → 4) and α-(1 → 6) linkages, beneficial for entrapment into on adsorption on the PUL structures. This entrapment and/or adsorption of genes prevent their degradation by the DNase degradation during the gene delivery applications (Ram Sarup Singh, Kaur, Hassan, & Kennedy, 2020; Ram Sarup Singh et al., 2015). Furthermore, it is possible to conjugate the PUL carriers with proper active targeting agents (e.g., antibodies, small molecules, aptamers) to provide targeted gene therapy strategies. Gupta et al. encapsulated pBUDLacZ plasmid into PUL NPs synthesized inside the aqueous droplets of w/o microemulsions. They reported that the synthesized NPs were spherical with 45 ± 0.80 nm diameter and exhibited high loading efficiency and sustained DNA release (Gupta & Gupta, 2004).

PUL conjugated with polyethyleneimine (PEI) is a hemocompatible component applicable for transferring a gene to liver cells and penetration of drugs into the cells (Rekha & Sharma, 2011). Previous studies demonstrated that PEI-PUL conjugating with siRNA might be applied for treating diseases such as cancer, dominant genetic disorders, etc. (D. H. Kim & Rossi, 2008; N. Zhang et al., 2009). For example, PUL was introduced into PEI for liver targeting in mice. It was shown that adding PUL to PEI significantly decreased mouse death after systemic injection. Indeed, after systemic injection, the PEI/fluorescein-labeled siRNA complex increased the level of fluorescence in the lung and the PEI-pullulan/siRNA complex led to an increased fluorescence level in the liver. The results proposed that the PEI-PUL may be a low toxic approach for the effective delivery of siRNA into the liver (Kang et al., 2010). Folate-PEI-PUL increased gene transfection efficiency and silencing effect. Such a system can be delivered via folate receptor-mediated endocytosis into FR-overexpressing cancer cells (J. Wang, Dou, & Bao, 2014).

In another attempt, cationized PUL (PUL-PEI) was synthesized and modified with ascorbic acid, an antioxidant molecule. It was shown that PEI-PUL modification with ascorbic acid can form nanoplexes with efficient cell internalization and transfection. An exciting feature of PUL–PEI–ascorbic acid (PPAA) that can be mentioned is, promoting collagen synthesis under influence of the ascorbic acid (Ambattu & Rekha, 2015). PEI-PUL was modified

with vinyl imidazole (PPI) displayed higher transfection efficiency than dextran PEI imidazole (DPI) due to the flexible nature of PUL (Diana & Rekha, 2017). Cationized derivatives of PUL can be fabricated through the incorporating of the thiol group via conjugating with protamine, as well as inserting the diethylamino ethyl amine (DEAE) to PUL backbone, i.e., DEAE PUL (Priya, Rekha, & Sharma, 2014; Ram Sarup Singh et al., 2015). San Juan et al. developed a tubular cationized -PUL hydrogel 3D structure to retain plasmid DNA and to deliver genes to vascular muscle cells or local arteries along with protecting from DNase degradation (San Juan, Ducrocq, et al., 2007). Another kind of cationic PUL prepared by a conjunction of PUL and spermine (spermine-PUL) demonstrated that it could hand over notch intracellular genes and have protective roles to release dopamine treatment of Parkinson disease (Nagane, Kitada, Wakao, Dezawa, & Tabata, 2009). As a multifunctional polymeric system, Priya and Rekha reported that thiolated cationic PUL could simultaneously deliver the p53 gene to the C6 glioma cells and increased drug retention that could be beneficial in improving the overall efficiency of chemotherapy (Priya & Rekha, 2016). In another study, cationized dextran and PUL which are modified with diethyl aminoethyl methacrylate (DEAEM) were used as vector backbones to create polymeric vectors (Sherly, Rekha, & Harikrishnan, 2020). Due to the definitive effect of PUL-g-poly(L-lysine) (J. S. Park et al., 2012a), PUL-protamine (Priya et al., 2014) and succinylated PUL (Hyemin Kim & Na, 2010) as PUL derivatives on decreasing cytotoxicity. Table 7 summarizes the studies that applied PUL and its derivatives as the carrier for gene delivery applications.

Table 7. Studies evaluated PUL and its derivatives as the carrier for gene delivery applications.

Pullulan derivatives	**Structure**	**Particle size (nm)**	**Gene (Gene/pullulan: µg/mg)**	**Target organ/cells**	**Model**	**Ref.**
CHP	Nanogels	4-17	Insulin 250/5	Primary cortical neurons and N9 microglial cells	*In vitro*	**(Morimoto, Endo, Iwasaki, & Akiyoshi, 2005)**
Cationized pullulan	Tubular hydrogels	200-1000	pSEAP 40/60	Vascular smooth muscle cells	*In vitro*	**(San Juan, Ducrocq, et al., 2007)**
	Matrices	200-1200	pSEAP 50/50	Vascular cells	*In vivo* and *in vitro*	**(San Juan, Hlawaty, Chaubet, Letourneur, & Feldman, 2007)**
	Hydrogels	N.S.	siRNA	Arterial wall	*In vivo* and	**(San Juan et al., 2009)**

					in vitro	
	Complex	N.S.	pCMV-p53/pCMV-βgal	T24 cells of human bladder cancer	*In vitro*	**(Kanatani et al., 2006)**
	Complex	350	Plasmid DNA	Sensory neurons	*In vitro*	**(Thakor, Teng, & Tabata, 2009)**
	Nanoparticles (NPs)	200-300	pCI-^{e}NICD	Bone marrow stromal cells	*In vitro*	**(Nagane et al., 2009)**
	Nanoplexe	97-222	p53	C6 glioma cells	*In vitro*	**Ambattu & Rekha, 2015**
PEI-pullulan	NPs	<200	siRNA	Liver	*In vivo*	**(Kang et al., 2010)**
	NPs	68-70	pGFP and pGL30.32/10	HepG cancer cells	*In vitro*	**(Rekha & Sharma, 2011)**
PGPLL	NPs	60-500	GFP plasmid DNA	HEK293, HepG2 and KB cells	*In vitro*	**(J. S. Park et al., 2012b)**
PPF	Polyion complexe	N.S.	DNA, siRNA	HeLa and HepG2 cells	*In vitro*	**Wang, Dou, & Bao, 2014**
PAEP	Conjugate	140-240	Plasmid DNA expressing green fluorescent protein (pEGFP)	Liver	*In vivo and in vitro*	**Liu et al., 2014**
CAPL	Nanoparticle	120-131	Tetramethyl rhodamine-labeled DNA (TAMRA-DNA)	HepG2 cells	*In vivo*	**Zhang et al., 2018**
PS	Magnetic nanoparticles	172±73	p53	Malignant glioma (U87) cells as glioblastoma cells	*In vivo*	**Eslaminejad, Nematollahi-Mahani, & Ansari, 2016**
Aminated pullulan	Nanogels	~155	miR-155-5p	Human umbilical vein endothelial cells (HUVECs)	*In vivo*	**Moraes et al., 2021**

Abbreviations: N.S.: Not specified; CHP: Cholesterol bearing pullulan; PS: Pullulan-spermine; PGPLL: Pullulan-g-poly(L-lysine); PEI: Polyethyleneimine; PPF: Polyethyleneimine pullulan folate; PAEP: Poly (β-amino) ester pullulan; CAPL: Charge-reversible pullulan derivative.

8.3.2. Yeast β-glucan

Yeast βG has been extensively studied for its immunostimulatory and immunomodulatory potential in the immune system (Geller, Shrestha, & Yan, 2019; Yasuda, Ogushi, Nakashima, Nakano, & Suzuki, 2018; Zhu et al., 2016). Yeast βG specifically binds to Dectin-1 and toll-like receptors expressing antigen-presenting cells, some T cell subsets, etc., and internalizes into these cells through the Dectin-1 receptor (Bastos et al. 2022; Alexander, Fiering, Ostroff, Cramer, & Mullins, 2018). βG particles of baker's yeast (*S. cerevisiae*) cell wall feature a hollow, porous

spherical structure which can be a carrier of therapeutic gene/agents to immune cells, increasing cellular uptake via receptor-mediated endocytosis (Sabu, Mufeedha, & Pramod, 2019). Also, βG particles of baker's yeast can be applied as a DNA/RNA delivery system for cell transfection (Sabu et al., 2019). For example, amphipathic siRNA–Endo-Porter complexes entrapped with βG from baker's yeast shells were developed to simplify siRNA delivery to targeted phagocytes. Endo-Porter was used to anchor siRNA inside glucan particles and facilitate siRNA escape from phagosomes (Tesz et al., 2011).

Recently an oral gene delivery system based on yeast cell wall particles and nanotube has been constructed to treat post-traumatic osteoarthritis (PTOA). Such a nanotube-RNA delivery system improved the sign of degenerative diseases due to some properties, including successfully internalized by macrophage, regulated gene expression (miR365 gene), and resisted against enzymatic degradation (L. Zhang et al., 2020). Similar release features have been observed by Aouadi et al. for oral delivery systems. They found that micrometer-sized β1,3-D-glucan from the baker's yeast mediated the oral delivery of siRNA directed against TNF-α for PTOA therapy and chronic diseases such as rheumatoid arthritis and atherosclerosis and type 1 diabetes treatment (Aouadi et al., 2009).

8.3.3. Non-yeast β-glucans (βGs)

Some βGs such as SPG form a triple helix in a neutral solution, while it changes to single chains in an alkaline solution. The single chains can re-form triple helix in a neutral solution (Yuting, Chen, Yang, & Cheung, 2020). During the process, they can form a stoichiometric complex with certain homopolynucleotides such as poly(dA) or poly(C) containing two main-chain glucans and one nucleotide base via hydrogen bonding and hydrophobic interactions (Sakurai, Mizu, & Shinkai, 2001; Sakurai & Shinkai, 2000). Trifluoroacetic acid treatment lowers the size of βG to nanoscale without affecting the properties of functional groups, and it performs well as a single-strand DNA carrier (Hwang, Lee, Gilad, & Choi, 2018). As nanosized βG carriers may be more efficient in genetic material transfer, siRNA encapsulated in βG nanoparticles (GluNPs) were designed and exhibited outstanding performance in gene delivery (K. Lee et al., 2020).

Antisense MIF-SPG complex can remarkably ameliorate inflammatory bowel disease (IBD) by suppressing MIF production in macrophages (Takedatsu et al., 2012). The advantages of the complex are its stability, resistance to deoxyribonuclease, and effective internalizing into macrophages through βG receptor Dectin-1 (Takedatsu et al., 2012). Previous studies demonstrated that complex of SPG with antisense oligonucleotides (AS-ODNs) (Izumi et al., 2016; Mochizuki & Sakurai, 2011) or siRNA (Mochizuki, Morishita, & Sakurai, 2013; Q. Zhang et al., 2015) with

attached 40-mer dA(dA40) could be taken up into cells expressing Dectin-1 such as macrophages and dendritic cells and efficiently silences genes in animal models of hepatitis and IBD (Takedatsu et al., 2012). After endocytosis through Dectin-1, AS-ODNs escaped from endosomes to the cytoplasm and hybridized with target mRNAs (Fujiwara, Izumi, Morimoto, Sakurai, & Mochizuki, 2019). Besides antigen-presenting cells, Dectin-1 is expressed on lung cells (Heyl et al., 2014); therefore, SPG complex can be applied for delivering AS-ODNs to silence gene expression in lung cancer cells (Izumi et al., 2016).

Recently, the SPG-antisense tumor necrosis factor α (TNF-α) complex, which was applied in a dextran sodium sulfate-induced colitis mouse model, showed high uptake into a macrophage, inhibition of TNF-α production, and ameliorated intestinal inflammation (Sakisaka et al., 2020). Lentinan, another fungal βG, is capable of binding to poly(dA) firmly. CpG DNA–poly(dA)/LNT complex has been developed based on intracellular cleavage of the disulfide bonds in response to reduction agent. According to the results, lentinan can be a good candidate for gene transfection (Liu et al., 2014).

9. Conclusions and perspectives

Many fungal strains capable of producing functional EPSs have been reported so far. Despite the benefits of these functional EPSs, limited studies on their commercial use have been investigated so far. Therefore, the present review explores functional fungal EPSs concerning their chemical modifications, wound healing properties, etc., while additionally highlighting the need for more work in the area, in vivo experiments, and clinical trials for an enhanced understanding of the bioactivity of fungal EPSs. There is also the need to improve EPSs yield via bioreactor design, process optimization, and analysis of the regulatory network of polymer biosynthesis. The current review also acknowledges that biomaterials based on fungal EPSs, e.g., PUL, Scl, SPG, LAS, can be employed in various biomedical fields such as TE, drug, and gene delivery.

Credit authorship contribution statement

Masoud Hamidi: Conceptualization, Writing – original draft, Writing – review & editing, Visualization, **Oseweuba Valentine Okoro, Peiman Brouki Milan, Mohammad Reza Khalili,** and **Hadi Samadian**: Writing-original draft, Writing –review & editing, **Lei Nie:** Writing –review & editing, **Amin Shavandi:** Writing – review & editing, Visualization.

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Declaration of Competing Interest

We declare that there are no conflicts of interest involved in this work.

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